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BIOCHEMICAL ANALYSIS OF MUTANTS: AN EMPIRICAL EXPERIMENT ON *LENS CULINARIS L.* SEEDS

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ABSTRACT

The present study is regarding Biochemical Analysis of Mutants of *Lensculinaris*.*Lens culinaris*, a leguminous plant and a member of pulse group. The use of pesticides such as-Monocrotophos, Endocel and Dimecron attributed to the toxic effects like inhibition of cell division, the increase in seedling mortality due to the disturbances at biochemical and cytochemical levels, which in turn cause chromosomal aberration, formation of laggards in the chromosome and extra-chromosomal injury. In this paper, effects of the aforesaid pesticides (Monocrotophos, Endocel and Dimecron) on mitotic and meiotic behaviour as well as polygenic traits has been analyzed. The frequency of chlorophyll mutations percentage and types of mitotic and meiotic aberrations, pollen sterility and polygenic variation with special reference to height of the plant, number of branches, flowering days, number of pods and seeds and seed weight. The present study also focuses on cytogenetic and mutational studies based on laboratory and field research.

KEY WORDS: Biochemical, Biometric Genetics, Cytotaxonomy, LensCulinaris, Cytochemical, Cytogenetic, Chromosomal aberration

INTRODUCTION

Lens culinaris, a leguminous plant and a member of pulse group, is a major source of protein, which is the most essential requirement of life. Studies in the five different varieties of *Lens culinaris* L. in the present investigation has amply established that pesticide treatment has affected the morphological, physiological and biochemical characters of the plants and brought about genetic variability probably due to gene mutation. On the whole five different types of mutants namely, Chlorophyll mutants (Leaf mutants),Dwarf Mutants, Sterile mutants, Unbranched and Erectmutants and Early maturing mutants have beenscreened out described earlier in section i.e.Morphological Studies. Due to comparatively lowerseed setting in chlorophyll mutants and dwarf mutantsas well as inconsistency in hereditary behavior ofUnbranched & Erect mutants and sterile mutants, it wasdecided not to carry out extensive biochemical studiesfor these mutants. On the contrary, the so called early maturing type of mutants have reflected comparativelybetter yield potential and early maturation of the plant.Naturally they may possibly lead toward someimportant commercial varieties. Therefore, only theearly maturing type of mutants have been selected inthe present work for biochemical studies, namely, totalprotein, protein sub-fraction, amino acid contents and SDS-PAGE profile studies.

OBJECTIVE:

To analyze the biochemical properties of mutants of *Lens Culinaris L.* Seeds.

EXPERIMENTAL MATERIAL AND METHODS:

The material used here was *Lens Culinaris* obtained from Rajendra Agricultural University, Pusa (Bihar). The seeds of all the varieties of *Lens culinaris*has been soaked in water and has been then allowed to germinate in separate Petri dishes on moist cotton and filter paper at 21°c ± 2°c. The seedlings have been transferred to earthen pots filled with soil and low proportion of sand for porosity. The plants have been kept under natural conditions.Floral buds of different sizes have been collected randomly and has been fixed in aceto-alcohol (1:3) containing a drop of ferric chloride for 24 hrs. It was further transferred in 70 per cent alcohol for preservation.

The Pesticides used in the research program namely:

(a). Monocrotophos, (b). Endoceland (c). Dimecron has been obtained from the various commercial sources

The morphological characters have been studied by visiting the experimental field regularly. Data has been collected on variants such as: (i) Early maturity, (ii) Flowering time, (iii) Sterility and (iv) Seed characters etc. and for the evaluation of M_1 and M_2 generations.

During the course of present research program, endeavors has been made to look into the details of the mutagenic effects of three Pesticides Monocrotophos, Endocel and Dimecron on some biological parameters of *Lens Culinaris*.The study has been focused on cytogenetic and mutational studies based on laboratory and field research.

Parameters of present study are:

(a) Percentage and time taken of germination
(b) Seedling survival
(d) Percentage of mitotic and meiotic aberrations
(e) Effects on plant attributes
(f) Chlorophyll mutation
(e) Biochemical analysis of mutants

RESULTS

The table incorporates observations on average yield per plant, shows higher yield in all the mutants as compared with their respective controls in both M_2 and M_3, generations. An average increase for yield per plant has been observed at lower doses of 0.10% & 0.25% treatment during the present investigation which has been discussed in Morphological Studies section (Table 1,2&3). However, performance of mutants has been almost uniform in both the generations.

Table- 1: REM values of different Bands of the albumin sub-fraction during SDS-PAGE in normal and treated population of Lens culinaris L.
E. LSG-8: M_1 Generation

	DOSES						
S.No.	*Control*	*0.10%*	*0.25%*	*0.50%*	*1.00%*	*1.10%*	*1.25%*
1.	11.5	10.5	11.0	11.5	11.0	11.5	11.0
2.	17.5	17.5	17.5	17.0	17.5	17.5	17.5
3.	22.5	22.5	22.5	22.5	22.5	22.0	22.0
4.	30.0	30.0	29.5	29.5	30.0	29.5	30.0
5.	43.5	43.5	43.5	44.0	43.5	44.0	
6.	55.0	54.5	54.5	54.5	54.5	54.5	55.0
7.	60.0	60.0	60.5	60.0	60.0	60.5	60.5
8.	63.0	63.0	63.0		63.0	63.0	
9.			----------	64.0			64.0
10.	66.5	66.5	67.0	66.5	66.5	66.5	66.5
11.			----------			69.0	69.0
12.	70.0	70.0	70.0	70.0	70.0		
13.	73.0	73.0	73.0	73.0	73.0	73.0	72.5
14.	77.0	77.0	77.0	77.0	77.0	77.0	77.0
15.	79.0	79.5	79.0	79.0	79.0	79.0	79.5
16.			----------	81.5	82.0	82.0	82.0
17.	83.0	83.0	83.0	-			
18.	85.0						
19.	88.0	87.0	88.0	87.5	87.5	87.5	87.5
20.	91.5	91.0	91.0	91.0	91.0	91.0	91.0
Total Bands 17		16	16	16	16	16	15

Sourse: Singh, 1993 (Unpublished Thesis)

Table: 2 REM values of different Bands of the albumin sub-fraction during SDS-PAGE in normal and treated population of Lens culinaris L.
E. LSG-8: M, Generation

DOSES

S.No.	*Control*	*0.10%*	*0.25%*	*0.50%*		*1 . 0 0 %*	
1.	*11.0*	*11.0*	*11.0*	*11.0*	11.0	11.5 17.0	11.0 17.0
2.	17.0	17.0	17.0	17.0	17.0	22.5	22.0
3.	22.0	22.5	22.0	22.0	22.5	29.5	30.0
4.	30.0	29.5	30.5	30.0	29.5	43.5	42.5
5.	43.5	43.0	43.5	43.0	42.5	55.0	55.0
6.	54.5	55.0	55.5	54.5	54.5	60.5	61.0
7.	60.5	60.5	60.5	60.5	60.5	-	-
8.	62.5	-	63.0	-		-	-
9.	-	-	-	64.5	64.5	66.5	66.5
10.	66.5	66.5	66.5	66.5	66.5	69.5	69.5
11.	70.0	69.5	70.0	70.0	70.0	73.5	73.0
12.	73.0	73.0	73.5	73.0	73.0	77.0	77.0
13.	76.5	77.0	77.0	76.5	77.0	80.0	79.0
14.	79.0	79.5	79.5	79.5	79.5	83.5	83.5
15.	83.5	83.5	83.5	83.0	83.5	87.5	87.5
16.	87.5	88.0	88.0	87.5	87.5	91.0	91.0
17.	91.0	_91.0	91.0	91.0	91.0		
Total Bands 16		15	16	16	16	15	15

Sourse: Singh, 1993 (Unpublished Thesis)

Table: 3 REM values of different Bands of the albumin sub-fraction during SDS-PAGE in normal and treated population of Lens culinaris L.
E. LSG-8:M_3 Generation

DOSES

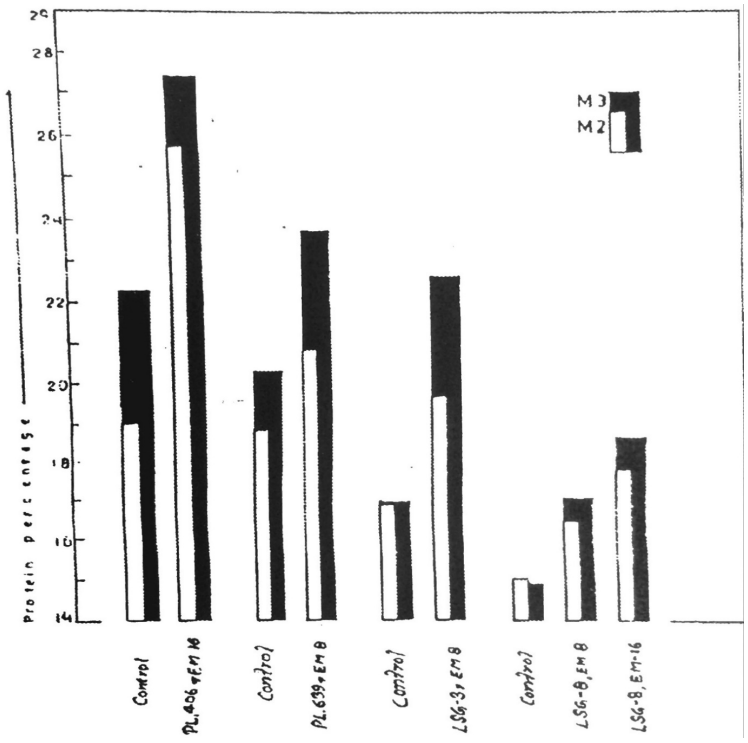
S.No.	*Control*	*0.10%*	*1125%*	*0.50%*	*1.00%*	*1.10%*	*1.25%*
1.	11.0	11.0	11.5	11.0	12.0	11.0	11.0
2.	17.0	17.0	16.5	16.0	17.0	17.0	17.0
3.	22.5	22.5	22.5	22.0	22.5	22.0	22.0

4.	30.0	29.5	29.5	30.0	30.0	30.0	31.0
5.	43.5	43.0	43.0	43.0	43.5	43.0	44.0
6.	55.0	55.0	54.5	55.0	54.5	55.0	55.0
7.	57.5	58.0	57.5	57.5	57.5	58.0	
8.	61.0	61.0	60.5	60.5	60.5	60.5	60.5
9.	----	63.0	62.5	63.0	63.0	63.0	62.5
10.	66.5	66.0	66.5	66.5	67.0	66.5	66.5
11.	70.0	70.0	70.0	70.0	70.0	70.0	70.0
12.	73.5	73.5	73.0	73.0	73.0	73.5	73.0
13.	77.0	77.5	77.0	77.0	77.0	77.0	77.0
14.	79.0	80.0	79.5	80.0	79.5	79.5	79.5
15.	83.5	83.5	83.5	83.0	83.0	83.5	83.0
16.	87.0	87.5	88.0	87.0	87.0	87.0	87.0
17.	90.5	91.0	9i)45	90.0	90.5	91.0	91.0
18.					92.5		
Total Bands 16		17	17	17	18	17	16

Source: Singh, 1993 (Unpublished Thesis)

TOTAL SEED PROTEIN

The protein contents (%) of respective controls and "Early Maturing" type of mutants of different varieties observed during present investigation in both M_2 and M_3 generations are presented in (Figure 1).



Source: Singh, 1993 (Unpublished Thesis)

Figure 1: Histogram showing a comparative account of total protein (in %) in control and mutant of lens culinaris in M2 and M3 Generations.

It reveals a remarkable increase in protein contents in different types of mutants, namely, M-EMJ6, PL.639 - EM8, LSG-3 - EM8, LSG-8-EM8 and LSG-8- EM16 in both the generations as compared to their respective controls.

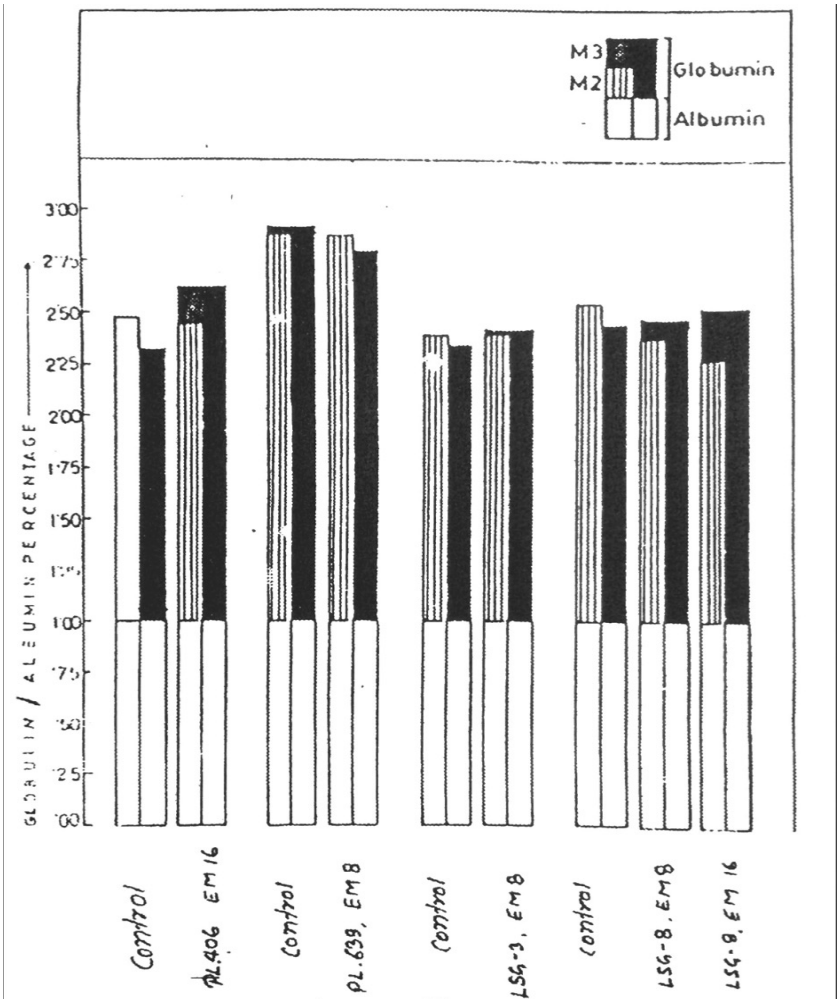
The highest percentage of soluble protein (25.74% & 27.38-%) has been noticed in mutant M-EM16 in both the generations, respectively as compared with 18.98% and 22.25% in that of consecutive generations of controls. On the basis of observed percentage increase in protein contents, the mutants may be arranged in decreasing order like, MEM16 - PL.639 - EM8 - LSG-3 - EM8 - LSG-8 -EM16 -LSG-8 - EM8. The comparative lower degree of range of variability in $\mathbf{M_3}$ generation as compared to M_2generation, suggests the stabilizing nature of themutants.

PROTEIN SUB-FRACTIONS (OSBORNE'S SOLUBILITY FRACTION)

The mean value of protein sub-fractions, namely, albumin, globulin, glutelin and prolamin in mutants and their respective controls both in $\mathbf{M_2}$ and $\mathbf{M_3}$generations are presented. The mean values at $\mathbf{M_2}$ and $\mathbf{M_3}$ generation though variable in mutants of all the varieties, reflect simultaneous changes in gene(s) controlling the manifestation of each sub fractions. A general increase in percentage of sub fractions of protein in mutants as compared with that of respective controls has been observed. The globulin has been found to be the major sub fraction followed by the albumin in all the mutants like their respective parental varieties. The variations for glutelin and prolamin sub fractions, has also been observed. For example, in PL. 406 control, glutelin percentage has been found to be lower than the prolamin whereas in mutant M- EM16, a slight increase in glutelin is obvious as compared with the control. The differential effect for the prolamin and glutelin sub fractions may be ascribed due to differential effect of mutation for these biochemical traits in the varietiesconcerned.

Figure 2 illustrates that the ratio of globulins/ albumins in mutants has considerably been altered as compared to their respective controls (PL.406-1.47:1 &1.3:1, PL. 639- 1.87:1 & 1.93:1, LSG-3-1.40:1 & 1.35:1

and LSG-8-1.54:1 & 1.46:1). Since albumins are richer in Sulphur-containing amino acids such as methionine and cysteine, such quantitative increase in albumins specially, may be an indication for the qualitative enhancement of amino acids.



Source: Singh, 1993 (Unpublished Thesis)

Figure: 2 Histogram Showing influence of Pesticide on Globulin/Albumin ration in control and mutant of Lens Culinaris L. in M_2 and M_3 Generations.

AMINO-ACID CONTENTS

The amino acid contents, as depicted in shows the similar trend as revealed by the protein contents. All of the mutants appear to have higher amino acid contents (mg/gm of seed meal) than their respective controls. As the amino acids are the building units of proteins so naturally any qualitative and quantitative alteration in amino acids will be reflected by the proteins.

The results obtained from mutant's biochemical -analysis in respect of total proteins, sub fractions of proteins and total amino acid contents, suggest that a concurrent increase in amino acid contents and major sub fractions of protein is responsible for increase in total seed proteins in the mutants isolated. The superiority of protein in mutants over their respective parents may also be ascribed by the findings.

SDS-PAGE BAND PROFILE IN MUTANTS:

The electrophoretograms of buffer extractable polypeptide (albumin) of both mutants and their respective controls have been illustrated in and their respective REM values on (Tables 4).

Table- 4Comparative REM value of different BANDS in Mutants with respective Mean Control in different varieties of Lens Culinaris L. during SDS-PAGE.

A. PL. 406 EM 16:			
S.No.	*Mean Control*	$^{M}2$	*Mutants* M_3
1.	10.4	10.0	10.5
2.		16.5	16.0
3.	17.1		
4.	22.2	22.0	22.5
5.	26.8	26.0	26.5
6.	30.0		
7.		32.0	32.0
8.		36.5	36.0
9.	38.5		
10.		440.2	40.2
11.	46.4		45.8
12.		52.5	
13.	55.0		54.5
14.		56.5	57.0
15.	58.5		
16.		60.0	60.0
17.	61.3		
18.	63.5	63.7	67.7
19.	66.8		
20.		67.5	67.5
21.		69.5	70.0
22.	70.8		
23.	73.5	73.5	73.5
24.	75.5	75.5	75.9
25.	77.5	77.5	78.0
26.	79.6	80.2	
27.	81.5		81.0
28.	83.7	83.5	83.5
29.	85.5	85.5	86.0
30.	87.9		91.2
31.	91.0		91.2
32.		92.5	

Total Bands.	22	21	21

Source: Singh, 1993 (Unpublished Thesis)

The mean REM values presented in the first column of the Tables 4 are the mean values of the REMdata for the respective controls of the M_1, M_2 and M_3generations of the corresponding variety, i.e. data of, last column. These values of mean REM positions have been depicted schematically in as the first schematic electrophoretogram preceding those of M_2 and M_3 generations of each mutant variety and is considered to be the "Control Band" for the respective mutants. This schematic electrophoretogram from mean REM data may be called hereafter, as "Mean Control" for the respective mutant. The boundaries of different bands of such controls inare on the error limits of the correspondingmean REM values. Clear difference in the SDS-PAGEband patterns in mutants as compared to their respectivecontrols are perceptible in spite of the fact that some ofthe electrophoretic bands are common to all mutantsand their respective controls.

No significant difference for the total number of bands present, has been observed in the M_2 and M_3generations of mutants as compared to the "Mean Control". However, highly significant changes in REM values and different band patterns have been observed during this experiment. A few of the bands of mutants, either seem to be newly appeared or migrated to new positions.

M-EM16

Electrophoretograms of M_2 and M, generations of possible mutant of PL. 406, namely M- EM16 are given together with the corresponding *"Mean Control"* in the figure. REM values obtained for the bands of M_2 and M_3 generations of M- EM16 from this figure is alsopresented in Table 4.Bands with REM values 16.5, 32.0, 36.5, 40.2, 56.5, 60.0, 67.5 and 69.5 in M_2 and M_3 generations seem to be slightly migrated or in few cases new appearances. The bands with REM values 80.2 & 92.5 in M_2 seem to be migrated to REM 81.0 and 91.2 in $M_{3/}$ respectively. However, a new weak band at REM 44.8 has been observed in M, generation, which remained absent in M_2 generation as well as controls (Table 4). Some of the bands of controls e.g. at mean REM 38.5 and 61.3 have been found to be disappeared or migrated in $M_2$and M, generations whereas the bands with REM 55.0 and 81.5 in controls have been found missing in M_2 but reappeared in M_3 generation. The results of Table 4show a clear-cut difference in band patterns of mutants compared to that of the control. Nevertheless, the band patterns of mutants in M_2 and M_3 generations are nearly similar with a slight variation.

Pl.639-EM8

Remarkable variations found in theelectrophoretograms of M_2 and M_3 generations of the possible mutant of PL. 639, namely PL. 639-EM8 together with the corresponding "Mean Control" are given in Figure-B24 and respective REM values. The slow-moving band having higher molecular weight with REM value 10.5 in control, has been found to migrate to 12.5 and 12.0 in the mutants, both in M, and M,, generations, respectively. The bands with REM value 63.5 seems to migrate to REM value 62.3 in M_2/which has again retained its originality in M, generation. A case of migration from REM value 85.8 in control as well as M to REM value 86.8 in M_3 is also evident for. The band of REM 92.5 in "Mean Control" has disappeared in M_2 but reappearedin M_3 generation of mutant. LSG-3-EM8.

Discernableband profile in controls as well as mutants ofLSG-3 namely, LSG3-EM8 in M_2 and M_3 generations. The band at REM 30.3 in "Mean Control" has been unaffected in M_2 but moves to REM 28.0 in M_3. Next, a new band at REM 42.0 and 85.0 both in M_2 and M_3 comes up. The bands with mean REM values 54.7,66.5 and 83.3 in control are found to migrate slightly to higher or lower REM values, bother in M_2 and M_3 generations of the mutants. The band with REM value 77.0 in M_2 generation of mutant seems to migrate to REM 78 in M, generation. Similarity between band profiles of M_2 and M, generation of mutants show recovery from pesticide treatment as well as stabilization tendency in M_3 generationof the mutant.

LSG-8-EM8 & LSG-8EM16

The electrophoretograms of polypeptide band patterns of M_2 and M_3 generations of the possible mutants of LSG-8, namely, LSG- 8-EM8 and LSG-8-EM16 are given together with the respective "Mean Control" in. REM values obtained from this figure for the bands of M_2 and M, of LSG-8-EM8 and LSG-8-EM6 respectively.The characteristic features of mutant LSG-8-EM8 is the presence of a new band at REM 75 in both M_2 andgenerations of mutants and a significant migration of band with REM 22.3 to highly stained bands at REM25.5 in both M_2 and M_3 generations. The bands with REM 59.5 and 72.6 in M_2 and M_3 seem to be migrated from their positions in control.

Similarly, few, characteristic band have also been found in case of LSG-8-EM, 6 mutants. A major migration of the first band at REM 11.3 in "Mean Control" to REM 14 in M_2 and M_3 generations of this mutant has been observed. A band at REM 54.8 in the "Mean Control" is found to migrate to REM 53 in the $M_2$and M, generations of the mutant. The band with REM 43.5 in the "Mean Control" is unmoved in M_2 but moves to 42 in M_3 generation. There is a new appearance of band at REM 47 in M_2 and M_3 generations. A few other of the weak bands have either appeared or disappeared or moved slightly in M_2 and M_3 generations of the mutants.

RELATIONSHIPS OF MUTANTS WITH PARENTAL VARIETIES

A new parameter, namely "Co-efficient or Difference" has been introduced below in order to study the intergeneration relationships of the controls as well as the relationship between a particular mutant generation and either control or another generation of the mutant. In order to calculate the co-efficient of difference (COD), firstly the pair of the electrophoretograms for comparison is selected. 'T_1' is the total number of bands of the first electrophoretogram and 'T_2' being that of the second. Total number of bands of the pair is then T_1,T_2.Next, the number of bands of the pair is found out which do not have common characteristics. This is done by counting all the bands that are missing or new bandswhich appear and giving this count, 'C_1', a weightage of one. When a definite case of migration of any band is established, its count,'C_2' is given an arbitrary weightage of two.The "Co-efficient of Difference"(COD) is then computed according to the following formula:

$$COD = \frac{T_1 + T_2}{T_1 + T_2 - C_1 - C_2}$$

Thus, in ideal case of one hundred percent similar pair of bands, C_1 and C_2 each will be zero hence COD will be equal to one. In a case of complete mismatch, i.e. only very small number of the band of one electrophoretogram coinciding with any band of the other electrophoretogram, COD will be very large.

In order to assess the applicability of COD parameter, it is first applied to compare the electrophoretogram of the controls of different varieties. Firstly, schematic electrophoretograms consisting of the mean REM positions of bands of M_1 M_2 and M_3 generations of each variety is constructed and presented. The band thickness in this figure representsthe limits of uncertainties of the mean REM values. Theschematic electrophoretogram thus constructed containsthe characteristics of all the three generations of aparticular variety including any minor genetic levelvariation or any environmental effect as well as effect ofthe experimental variations. This schematic electrophoretogram is therefore, called "Mean Control"of the respective variety.The bands of the mean control of one variety has been compared with the bands of the mean control of another variety and the corresponding COD value is calculated. For all the five varieties, there are a total of ten such inter- varietal COD values.

The minimum inter-varietal COD is 1.4 for LSG-3 Vs LSG-8 whereas the maximum COD is 2.6 for PL. 639 Vs LSG-10 it should be noted that a COD value of 2 will arise from about 50% even

minor or major mismatch of band patterns. From the Table-B50, it is apparent that neither of the COD values' is very close to unity nor any value is exceptionally high. This indicates to the inter-varietal diversity to somewhat moderate extent. The varieties appear to be close relatives, reflected from the mean COD value of 1.8 ± 0.3. For instance, in hypothetical case of such comparison with species ofLeguminaceae other than Lens culinaris L. should lead to values much higher than 2.6. Thus, the COD value is a good measure for comparison of electrophoretograms pairs which considers not only the similarity but also the differences between them.

Table 5 presents the result of COD value calculations from the comparisons of the electrophoretograms of mutant's generations, M_2& M_3, with the respective "Mean Controls" as well as those from the Comparisons of M_2 Vs M_3generations of mutants themselves. The COD values for comparison of Control Vs M_2, or Control Vs M_3, of mutants remain always higher as compared to COD for M_2,Vs M_3comparison of the mutants themselves.

Table 5 A comparative account of COD values between Mean Control and different generations of Mutants in five varieties of Lens culinaris L. during SDS- PAGE

	CV_SM_2	CV_SM_3	$M_2V_SM_3$
PL.406-$EM_1$6	1.7	1.3	1.2
PL.339-EM8	1.5	1.4	1.2
LSG-3-EM8	1.5	1.6	1.2
LSG-8-EM8	1.6	1.4	1.1
LSG-8-$EM_1$6	1.6	1.6	1.2

Source: Singh, 1993 (Unpublished Thesis)

For example, COD for M_2 VsM_3 for all the five mutant varieties vary between 1.1 to 1.2. Whereas on the average a value of 1.6 is found for COD values for any inter-varietal mutant comparisons or comparisons of M_2 or M_3 mutant with "Mean Control". Another important feature of COD values lies in the fact that mostly, control Vs M_2 COD data remains higher than Control Vs M_3 data. For instance, COD (Control Vs M_2) for M-EM 6 is 1.7. Whereas COD (Control Vs M_3) for M-EM 6 is 1.3. Thistrend is maintained for all the mutant varieties exceptLSG-3-EM8 which has the reverse order, COD relatingto M_2 is 1.5 whereas that relating to M_3 is 1.6.

Incidentally, COD data for comparisons among the two mutant varieties of LSG-8 are higher. The COD values for the comparisons of the mutant "M_2" of LSG8-EM8 with the mutant "M_2" or "M_3" of LSG-8-EM 6 are 1.9 and 2.0 respectively. On the other bands COD values for the comparisons of the mutant generationof LSG-8 EM8 with the mutant generation "M_2" or "M_3" or LSG-8-EM 6 are 1.5 and 2.0 respectively.

DISCUSSIONS

To mention a few salient features of this method, the protein and enzyme pattern variations among the varieties havebeen used by Abraham and Cherian, 1978 and Auerbach, 1962 in order to identify the various cultivars. Identification of cultivars based on morphological characteristics of plants or plant parts are subject to influence by pesticide treatment and environmental variation (Mohan, 1975). It has been realized that protein band patterns because of its unique and specific pattern and comparatively less influence of treatment and environmental variation can very well supplement the morphological trait in cultivar identification.

In the present experiment the varieties as well as different types of mutants isolated has been found to differ with regard to total number of band in a variety, total number of bands at a particular dose and in specific mutant with presence/absence of a particular band and its intensity. The cause of the presence of certain specific band in some varieties and at particular dose while its absence in others may be ascribed due to the fact that the gene responsible for the production of specific polypeptide might have mutated

resulting in complete loss of its activity in these genotypes (Sinha, and Acharia, (1972, 1975). Anotherpossibility of its absence in some varieties and presence in other may be due to the changes in regulatory gene which did not allow the gene for this particular band to be expressedin the varieties concerned, respectively (Vance, and Smith, 1962).

Similarly changes in the band patterns in tile treated populations as well as mutants have been confirmed to be the result of the alteration in polypeptides of seed protein due to gene mutation (Abo-Hegwzit, 1980). "The change in the mobility and intensity (REM values) of certain bands may be due to a decrease of the molecular weight of some subunits of the protein" (Rao, 1962).

The essential features of the similarities or differences of the electrophoretograms of the mutantsin M_2 or M_3 generations with the M_1,M_2 or M_3 generations of the controls of the respective varieties on simply the "mean Control" have been summarized in the form of "Co-efficient of Differences" data. It is worth to mention that the COD value incorporates the essential similarities in different varieties considered, between the pair of electrophoretograms as well as it has the information about the essential differences. The computation of COD values emphasizes the definite case of band migration for mutant variety identification rather than presence or absence of a particular band. Enhanced weightage for the case of migration of bands can be justified by the argument that a migrated band is theevidence of the change in the corresponding gene dueto which a particular band may show concomitantlyslow and fast electrophoretic mobility and ultimatelya result(Srivastava, 1979). For instance,"mutation by substitution in DNA" may lead to a small-scale change in the corresponding polypeptidesequence with a concomitant change in its molecularweight giving rise to a noticeable migration of thecorresponding gene. This view isgrip ported by Ahmed, M and Grant, (1972) who while working with hemoglobin molecules advocated that change in electrophoreticmobility may result from the substitution of even asingle amino acid, the altered electrophoretic mobilityreflects a change of the protein molecule. This ispossible when the substituted amino acid causes achange in charge and mass that is different from theone, it replaces. This change is caused by mutation instructural genes (Auerbach, 1961).

On the other hand, either disappearance of an existing band or appearance of a new band may be due to one or more of the following reasons. Firstly, absence of a polypeptide sequence due to mutation by dilationof DNA strand may cause the disappearance of the corresponding band. Alternatively, insertion mutation, on the similar grounds, may cause the appearance of a new band. Thirdly, there may be appearance/disappearance of bands of random nature, depending upon the concentration of concerned protein governed by the experimental error conditions.

It has been seen that inter-varietal COD data range 1.4 to 2.6, indicating a moderately closed relationship among the varieties under present studies. There is, however, no pair of varieties having very close relationship. For any two varieties or any two generations to be very close to each other, the corresponding COD values should be close to unity. Thus, the magnitude of the COD values translates the distance of relationship between the pair of comparison. With this parameter and from the data of Table 5, it is apparent that the mutants in the M_2 generation are sufficiently distantly related to the respective controls. For instance, COD of control Vs Mutant-M_2 comparison ranges from 1.5-1.7.

A slight trend of recovery although not very much obvious, is indicated by a slightly lower COD values in the third mutant generation (M_3) compared with the respective controls (Gupta, and Mallik, 1978). However, the trend of stabilization of mutants is confirmed by the very low COD values when mutant M_2 is compared with respective M_3, generation of the same mutant. In

other words, M_3 -mutant generation, reflected in thecorresponding COD values of 1.1 - 1.2 incidentally a case of very distant relationship between two of the mutant varieties has also been found. To mention it, COD for comparisons among mutant generations, of LSG-8-EM8 and LSG-8-EM16, remains high at about 2.0.

To sum up, it has been established that some of the mutant varieties identified on the basis of morphological, physiological and biochemical parameters, do have remarkable features in their electrophoretograms suggesting the existence of genotypically different varieties (Smith, 1996). The positive alteration in seed protein and amino acid contents, the changes in protein sub-fractions and the band patterns during SDS-PAGE and their heritable nature in the mutants, indicatethat possibly induced changes are a consequence ofmutated genes.

REFERENCES

1. Abo- Hegwzit, A.M.T. (1980). Seed protein and other characters in M4 generation of chickpea. Ind. J. Genetics and Plant breeding 40(i):122- 125.
2. Abraham and Cherian, V.D. (1978). Studies on cellular damage by extracts of betel leaves used for chewing. Cytologia 43: 203.
3. Ahmed, M and Grant, W.F. (1972). Cytological effects of the pesticides phosdrin and bladex on *Tradescantia* and *Vicia faba*. Can. J. Genet. Cytol. 14: 57-165.
4. Auerbach C. (1961). Chemicals and their effects. In: Symposium on Mutation and Plant Breeding, National Research Council Publication 891, 120-144.
5. Auerbach C. (1962). Mutation: An introduction to research on Mutagenesis. Part I. Methods. Edinburgh: Oliver & Boyd.
6. Gupta, V.K. and Mallik, S.S. (1978) Electrophoretic patterns among seed proteins from different Varieties of rice. Pantnagar J. Research, 3(1): 1-3.
7. Mohan, S.T. (1975). Cytological effects of fungicides plantvax and vitavax on somatic cells of *Allium cepa*. Curr. Sci. 44(22): 813-814.
8. Sinha, S.S.N. and Acharia, S.S. (1972) Karyotype analysis in some varieties of *Lens culinaris*. Cytologia 37: 637- 683.
9. Sinha, S.S.N. and Acharia, S.S. (1975) Meiotic analysis in some varieties of *Lens culinaris*. Cytologia 40: 269- 276.
10. Singh, Anita. 1993. Effect of Pesticides and biochemical studies in Some varitiesof Lens *culinaris*. B. S. R. B. Bihar University, Muzaffarpur. Unpublished Thesis.
11. Smith, K. (1996) Environment Hazards: Assessing risks and reducing disaster. Routledge Publ. London (Second Edition).
12. Srivastava, S. and Sarma, Y.G.R.K. (1979). Effects of two insecticides Dimecron and Nuvacronon the survival, growth and nuclear cytology of *Oedogonium gunnii* wittr. J.Cytol. Genet. 14:163-172.
13. Vance, B.O. and Smith, B.L. (1962) Effect of five herbicides on three green algae. Texas J. Sci. 20: 329- 337.

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