

LSD induced chlorophyll mutations in barley

M. P. Singh and C. S. Kalia

Division of Genetics, Indian Agricultural Research Institute, New Delhi-110012 (India), 22 February 1978

Summary. In earlier reports, LSD was found to induce extensive chromosomal aberrations in barley. With the same dose it is now observed that LSD is potent in the induction of chlorophyll mutations. This may be the first evidence that the drug induces gene mutations in plants.

There have been a number of reports that the psychotomimetic drug LSD (lysergic acid diethylamide) induces genetic damage, as shown by its effect in producing chromosomal aberrations in a number of test organisms, including *Drosophila*^{2,3}, mice⁴, *Antheraea eucalyptae*⁵, barley^{6,9}, rye⁸, onion and human leukocytes^{9,11}. No such aberrations, however, could be detected in some other materials such as *Vicia faba*¹², *Allium cepa*¹³, and human leukocytes¹⁴. The evidence for genetic damage has been based almost entirely on the induced chromosomal breaks with little or no evidence that the drug also induces gene mutations. It has yet to be unequivocally determined whether and in what circumstances this chemical is mutagenic.

Material and methods. In the course of the present study, seeds of diploid hulled barley variety NP113 (*Hordeum vulgare*, $2n=14$) were germinated overnight at $25\pm1^{\circ}\text{C}$. For each treatment and their corresponding control, 100 seeds of uniform size were selected. Following germination, the seeds with their emerging root tips, were treated with freshly prepared aqueous solution of LSD for 4 and 8 h. The drug sample was prepared by Sandoz Pharmaceutical, Hannover, New Jersey, USA. The germinated seeds of the control series were soaked in 50 ml of distilled water at 25°C for the duration of the 2 treatments described above.

Observations and discussion. The first generation (M_1) plants from the treated and the control seeds were raised in pots and were selfed to avoid possible outcrossing. M_2 progenies were raised from the single M_1 plants and their population was screened for chlorophyll mutations. A number of families were found to be segregating for normal and chlorophyll deficient type, which have been classified following the description given by Gustafsson¹⁵. The relative frequency and the spectrum of mutants is summarized in the table. It will be seen that both treatments are quite effective in inducing chlorophyll mutants of various types. It is interesting, however, that the nonviable type was recorded in the 8-h treatment, whereas viable types, i.e. *xantha-albina* and *maculata*, were randomly distributed in both sets of treatments. Screening of M_2 populations along with their respective controls was undertaken in the field under natural conditions of temperature and light. These factors are considered most influential¹⁶ in determining the optimum colour expression. Hence the possibility of losing some lethal *albina* and *xantha* seedlings in the initial stages of germination cannot be excluded. 3 typical mutants were carried forward in M_3 ; 2 bred true and the 3rd segregated. Chromosomes were not examined.

For all mutagens analyzed in barley¹⁷, the approximate proportion of *albina* (white) reaches 50%, *viridis* (lightgreen) 30% and *xantha* (yellow) 5%. It is reported that X-ray treatment induces *albina* mutation with considerably higher frequency when compared to different chemical treatments¹⁸. Originally, numerous gene loci were reported to be involved in barley with regard to the *albina* and *viridis* phenotypes, fewer for *xantha* and perhaps only a single gene locus for *albo-xantha*¹⁹. The spectrum with LSD, as evident in our observations, is strikingly unusual, because there is predominant occurrence of *xantha-albina* (base white - tip yellow) and *maculata* (irregular lightgreen or yellow patches) types which are considered comparatively rare. It appears that there is a tendency for a selective mutagenic effect of LSD which gives rise to non-random appearance of mutations. Longer duration of treatment gives a reduced percentage of segregating M₂ families (16.6 vs 36.4%) but increased mutation percentages (1.1 vs 0.8%), when scored on the basis of total M₂ population. The reduction in the number of mutated families might be accounted for by an accelerated process of recovery in the 8-h treatment, but the efficiency of the said treatment is, on the other hand, evidenced by the increased clustering of mutations in the affected progenies. Frequency of chlorophyll mutations in barley with different mutagenic treatments were reviewed and the range varied from 2.6 to 23.4% in the effective treatments²⁰. The percentage of LSD-induced chlorophyll mutations on the basis of M₂ populations screened was 0.95, thus indicating a weak mutagenic response, which could possibly be due to lower doses in our treatment. The earlier report on *Arabidopsis*²¹ showed no chlorophyll mutation, and in the fungus *Ophiostoma* LSD failed to produce any significant mutagenic effects. On the other hand, LSD has been reported to induce sex-linked lethals in *Drosophila*, when very high doses of 2000-10,000 µg/ml were given as intra-abdominal injections²³. Lower doses were found to be ineffective.

A study on meiotic chromosomes in LSD-treated diploid rye showed chromosomal abnormalities⁸, indicative of genetic repatterning in the treated material. For a given dose of LSD (30 µg/ml), the amount of chromosomal damage was significantly higher in onion than in barley or rye⁸, suggesting species-specific susceptibilities. In order to define threshold effects of LSD²⁴, the dose-effect relationship with different test materials should be further investigated.

From our observations it is evident that a low dose of 25

Frequency and spectrum of LSD-induced chlorophyll mutants in barley

Treatments	Percentage of segregating M ₂ families	Control/M ₂ population	Frequency of mutant plants in M ₂ <i>albina</i>	<i>xantha</i>	<i>xantha-albina</i>	<i>maculata</i>	Percentage of chlorophyll mutants
Control	0	2050	0	0	0	0	-
4-h LSD 25 µg/ml	36.3	3675	0	1	11	18	0.81
8-h LSD 25 µg/ml	16.6	2475	3	4	8	14	1.17
4-h + 8-h (Pooled/percentage)	26.5	6150	3	5	19	32	0.95

µg/ml, earlier found to be effective in producing chromosomal damage^{6,7}, is also capable of inducing chlorophyll mutations in barley. This finding seems to provide the first evidence that the drug induces gene mutations in plants. The mutagenic action of LSD could be due to its interaction with DNA²⁵, the mechanism of which is not clearly understood.

- 1 Acknowledgment. We are grateful to Dr H.K. Jain, Indian Agricultural Research Institute, for his interest and guidance.
- 2 L.S. Browning, *Science* 161, 1022 (1968).
- 3 E. Vann, *Nature* 223, 95 (1969).
- 4 N.E. Shakkebeck, J. Phillips and O.J. Rafaelsen, *Science* 160, 1246 (1968).
- 5 N.C. Quinn, *Entomol. News* 81, 241 (1971).
- 6 M.P. Singh, C.S. Kalia and H.K. Jain, *Science* 169, 491 (1970).
- 7 C.S. Kalia, M.P. Singh, H.K. Jain and R.K. Katiyar, *Chromosoma (Berl)* 32, 142 (1970).
- 8 R.S. Sadasiviah, G.B. Collins and D.L. Davis, *Chromosoma (Berl)* 44, 309 (1973).
- 9 M.M. Cohen, M.J. Marinello and B. Nathan, *Science* 155, 1417 (1967).

- 10 M.M. Cohen, K. Hershoom and W.A. Froese, *New Engl. J. Med.* 227, 1042 (1976).
- 11 M.J. Corey, J.C. Andrews, M.J. Meleod, J.R. Mclean and W.E. Wilby, *Science* 282, 943 (1970).
- 12 S. Sturelid and B.A. Kihlman, *Hereditas* 62, 259 (1969).
- 13 H.P. Riley and J.V. Neuroth, *J. Hered.* 61, 282 (1970).
- 14 D. Grace, E.A. Carlson and P. Goodman, *Science* 161, 694 (1968).
- 15 A. Gustafsson, *Lunds Univ. Arsskr.* 361 (1940).
- 16 R.A. Nilan, C.F. Konzak, R.E. Heiner and E.E. Froese-Gertzen, in: *Barley genetics*, vol. 1, 35. Wageningen, 1963.
- 17 R.A. Nilan, in: *The cytology and genetics of barley*. Monograph, vol. 3, 1964.
- 18 E.A. Favret, *Hereditas* 46, 622 (1960).
- 19 D. Von Wettstein, A. Gustafsson, L. Ehrenberg and F. Frosch, *Arbeitsgem. Nordrheinwestfalen* 73, 7 (1959).
- 20 T. Kawai, in: *Induced mutations in plants*, IAEA 137 (1969).
- 21 T. Mabuchi and T.J. Arnon, *Arabidopsis Inform. Serv.* 6, 27 (1969).
- 22 G. Zetterberg, *Hereditas* 62, 262 (1969).
- 23 Norman I. Dishotsky, W.D. Longman, R.E. Mogar and W.R. Lipscomb, *Science* 172, 431 (1971).
- 24 E.H. Markowitz, E. George, G.E. Brosseau and E. Marjowitz, Jr, *Mutation Res.* 8, 337 (1969).
- 25 T.E. Wagner, *Nature* 222, 337 (1969).

Influence du rang de ponte dans la transmission du caractère *atrophie gonadique* chez *Drosophila melanogaster*

Influence of the rank of egg-laying on the transmission of the *gonadal atrophy* character in *Drosophila melanogaster*

G. Periquet

Laboratoire de Génétique des Populations, Tour 42-32, 4^e étage, Université Paris 7, F-75221 Paris Cédex 05 (France), 17 mars 1978

Summary. Gonadal atrophy (*atrophie gonadique*, *ag*) is a hereditary character of variable expression and penetrance in *Drosophila melanogaster*, the penetrance of which varies with temperature but also with the rank order of eggs produced. It is shown that 'early' zygotes are more often affected than 'late' zygotes who not only, but also transmit the character more efficiently to their offspring.

Chez *Drosophila melanogaster*, le caractère *atrophie gonadique* (*ag*) se présente sous 2 aspects selon qu'une seule gonade est atrophiée (type S1) ou les deux (type SO). La fréquence d'*ag* augmente avec la température d'élevage, ce caractère manifestant une pénétrance variable en fonction de la température subie durant les 2 premières h du développement embryonnaire¹. L'atrophie gonadique peut être attribuée à une thermo-sensibilité des cellules polaires embryonnaires induisant leur dégénérescence², les imagos formés présentant en règle générale des gonades agamétiques³. Cette dégénérescence des cellules polaires apparaît déterminée par un système polyfactoriel composé principalement de 2 gènes ou groupes de gènes localisés sur les autosomes 2 et 3⁴.

La fréquence de ce caractère varie en fonction du rang de ponte, les œufs pondus durant les 2 premiers jours donnant naissance à des imagos plus fréquemment porteurs d'une atrophie des gonades⁵. Les travaux présentés ici cherchent à préciser la nature de cette différence en vérifiant si la transmission du caractère *ag* par des individus issus d'un lot d'œufs pondus durant les 2 premiers jours est identique à celle des individus issus d'un lot d'œufs pondus les jours suivants.

Matériel et méthodes. A partir de la souche Marseillan 65 conservée au laboratoire à 20 °C par repiquages massifs, plusieurs lignées ont été sélectionnées durant 5 générations de croisements frère × sœur à 25 °C, afin d'augmenter la

fréquence du caractère *ag*. C'est la lignée *Ma 65 A*, fournissant la meilleure réponse à la sélection (de l'ordre de 50% d'*ag* chez les femelles et 35% chez les mâles), qui a été utilisée.

2 séries d'expériences similaires ont été réalisées à 8 mois d'intervalle. Dans chaque cas, une quinzaine de couples est extraite de la souche *Ma 65 A* dès l'émergence. Placés chacun à 25 °C dans un tube de milieu au maïs (lot 1), ils y restent 2 jours au-delà desquels les mâles sont enlevés et les femelles transférées dans un tube de nourriture fraîche. Ce transfert est renouvelé tous les 2 jours jusqu'à ce que les femelles aient atteint l'âge de 8 jours (lot 2, 3, 4). Chaque population *F*₁ issue de ces différents tubes est repiquée et donne naissance à une population *F*₂ dont seront observés uniquement les individus émergés les 2 premiers jours.

Résultats. On trouve en *F*₁ une différence de fréquence des formes *ag* entre les imagos issus des œufs pondus les 2 premiers jours (Lot 1) et ceux issus des œufs pondus les jours suivants (Lot II = lot 2 + lot 3 + lot 4). Les différences sont significatives pour chaque expérience (tableau). En *F*₂, les différences entre les mouches issues des œufs pondus les 2 premiers jours et les autres sont beaucoup plus faibles et ne sont significatives avec le test du χ^2 que dans la première série d'expériences. Ceci peut s'expliquer par le fait que la souche *Ma 65 A* a évolué entre les 2 séries d'expériences: en effet, sélectionnée pour une forte fréquence de *ag* cette souche n'est pas stable et tend à retourner vers un état