Theor. Appl. Genet. 55, 253-255 (1979)

Organophosphate Induced Chlorophyll Mutations in *Hordeum vulgare*

B.B. Panda and C.B.S.R. Sharma
Cytogenetics Laboratory, Department of Botany, Berhampur University, Berhampur (India)

Summary. Ten Organophosphorus (OP) insecticides are tested for their genetic toxicology in the *Hordeum vulgare* system. Of these, 8 OPs induced chlorophyll mutations of which 6 are new discoveries.

Key words: Organophosphorus insecticides – Chlorophyll mutations – Genetic toxicology

Introduction

Environmental chemicals have been under screening for over a decade for their genetic toxicity in several test systems (Kilbey et al. 1977; Scott et al. 1977). Of these, pesticides have gained increasing importance, particularly since their teratogenic and carcinogenic effects have been found to be related to mutagenecity (Durham and Williams 1972). Organophosphate pesticides have only become a source of concern comparatively recently when some of them were reported to have an effect on the genetic material in different systems (Wild 1975). However, information from plant systems has been rather scanty even though the recognition of plants as monitors of genetic damage, especially from agrochemicals, has been on the increase (Zimmerman 1976; Nilan and Vig 1976; de Serres 1978). Therefore, an attempt has been made to test the mutagenic potential of some organophosphorus insecticides using *Hordeum vulgare* as the test system.

Materials and Methods

1500 ppm solutions of 10 organophosphorous insecticides, namely: Phosphamidon (Demecron®), Monocrotophos (Nuvacron®), Dichlorvos (Nuvan®), Trichlorfon (Dipterex®), Thiademeton (Disyston®), Oxydemeton methyl (Metasystox®), Fenthion (Lebaycid®), Fensulfothion (Dasanit®), Fenitrothion (Folithion®), and Methyl Parathion (Metacid®) were freshly prepared in phosphate buffer at pH 7. In the case of Fensulfothion the solution was further diluted to 500 ppm as germination was inhibited at higher concentrations. Active ingredients were always used. 150 barley seeds (*Hordeum vulgare* L. C.V. 292) were presoaked in distilled water for 15 hours and treated in test solutions for 6 hrs to coincide with peak DNA synthetic activity (Khalatkar 1976). During the treatment the flasks containing the seeds were periodically shaken for 2-3 minutes. As standards, positive and negative controls were maintained at the same time. Seeds treated in 1500 ppm of Ethyl methanesulfonate (EMS) served as positive controls. The negative controls were the solvent controls consisting of seeds treated in buffer alone or acetonised buffer, depending on the solubility of the test compounds.

After treatment the seeds were washed thoroughly in tapwater and germinated in petri-dishes on moist filter paper. 7 day-old seedlings were transplanted to the field and allowed to grow to maturity as M_1 plants. From the harvest of this crop 1000-2000 seeds were sown in sandbeds in greenhouses. The seedlings emerging from these M_2 seeds were screened for morphological and chlorophyll mutants following conventional practice (Gaul 1964; Wuu and Grant 1966). The treatments were carried out during November 1977 and the M_2 seedlings were screened in the following season, i.e. November, 1978.

Results and Discussion

A number of both morphological and chlorphyll-deficient seedlings were scored in all treated samples. These include: 1) coleoptile elongation without leaf emergence and 2) chlorophyll deficiencies. The former die out gradually and therefore were designated as lethals. These were produced by all the organophosphates tested. The chlorophyll deficiencies were: xantha, albina, alboviridis and viridoalbina. Of these, viridoalbina and albina were produced only by EMS and methyl parathion, respectively.

As stated above, lethals were produced by all the organophosphates tested here. Similar deformities were obtained in barley in response to some herbicides and were attributed to altered gene activities affecting enzyme pro-

0040-5752/79/0055/0253/$ 1.00

Table 1. Mutants in the M_2 seedlings of *Hordeum vulgare* induced by organophosphate insecticides

Organophosphate insecticide	Seedlings screened	Lethal	Xantha	Albina	Alboviridis	Viridoalbina	% total mutants	% chlorophyll mutants
Solvent control	934	4	–	–	–	–	0.43	0.0
Phosphamidon	1247	9	–	–	6	–	1.20	0.48
Monocrotophos	1342	4	–	–	7	–	0.82	0.52
Dichlorvos	990	4	–	–	8	–	1.21	0.82
Trichlorfon	1008	4	–	–	6	–	0.99	0.60
Thiodemeton	843	6	–	–	4	–	1.18	0.47
Oxydemeton methyl	719	8	–	–	1	–	1.25	0.14
Fenthion	880	7	–	–	–	–	0.80	0.0
Fensulfothion	1200	8	–	–	–	–	0.67	0.0
Fenitrothion	990	3	1	–	2	–	0.61	0.30
Methyl Parathion	1929	4	1	1	5	–	0.57	0.36
Ethyl methane sulfonate (Positive control)	969	6	6	–	6	3	2.17	1.55

duction and function and not to gene mutation per se (Wuu and Grant 1966). It appears to us that since defective enzymatic activity necessarily involves gene activity these deformities should be considered mutant, lethal though they are.

The chlorophyll mutants were well known from the literature concerned with plant mutations (Nilan 1964; Gaul 1964). Alboviridis has been produced by 8 OPs, xantha by 2 OPs, and albina by 1 OP only. Mutant yields were much lower than those obtained in the EMS solutions. Dichlorvos and Trichlorfon gave comparatively higher mutant frequencies followed by Monocrotophos, Phosphamidon, Thiademeton, Methyl parathion, Fenitrothion and Oxydemeton methyl, in descending order. No mutations were induced by Fenthion and Fensulfothion (Table 1).

Among the OPs currently tested chlorophyll mutations were first obtained by Blixt and Muller in *Pisum* (Lofroth et al. 1969) with Dichlorvos and by Wuu and Grant (1966) in Barley with Phosphamidon, while Bhan and Kaul (1975) failed to obtain any with Dichlorvos. A few carbamate herbicides were also known to induce similar chlorophyll mutations in Barley (Wuu and Grant 1966; Nasta and Gunther 1973). The ability of these OPs to cause chlorophyll mutations may be due to their DNA-alkylating properties. Dichlorvos, Trichlorfon, Oxydemeton methyl and Methyl parathion are known for this ability (Lofroth 1970; Bedford and Robinson 1972). However, the variations in the mutation frequencies may be a function of the relative efficiencies of the OPs to reach and/or act on the cellular target.

The present work confirmed the mutagenic potentials of Phosphamidon and Dichlorvos and reports for the first time the ability of six other OPs to cause chlorophyll mutations. It may be significant to note that besides serving as a monitor of genetic damage, the barley-assay projects the problem of accentuated variability by the agrochemicals into crop ecosystems.

Acknowledgement

The authors are grateful to Dr. D.C. Joshua of the Bhabha Atomic Research Centre, Bombay for the supply of the seed stocks and Dr. N.P. Sarma of the Central Rice Research Institute, Cuttack for the gift of EMS. Thanks are also due to Bayer (India) Ltd. and Ciba-Geigy Ltd. Bombay for the supply of the test compounds. The financial assistance to one of us (BBP) by Council of Scientific and Industrial Research, New Delhi is acknowledged.

Literature

Bedford, C.T.; Robinson, J.: The alkylating properties of organophosphates. Xenobiotica 2, 307-337 (1972)

Bhan, A.K.; Kaul, B.L.: Cytogenetic activity of Dichlorvos in Barley. Ind. J. Exp. Biol. 13, 403-405 (1975)

de Serres, F.J.: Introduction: Utilisation of higher plant systems as monitors of environmental mutagens. Environ. Health Persp. 27, 3-6 (1978)

Durham, W.F.; Williams, C.H.: Mutagenic, teratogenic and carcinogenic properties of pesticides. Ann. Rev. Entomol. 17, 123-148 (1972)

Gaul, H.: Mutations in plant breeding. Rad. Bot. 4, 155-232 (1964)

Khalatkar, A.S.: Influence of DMSO on the mutagenecity of EMS in Barley. Bot. Gaz. 137, 348-350 (1976)

Kilbey, B.J.; Legator, M.S.; Nichols, W.W.; Ramel, C. (Eds.): Handbook of Mutation Test Procedures. Amsterdam: Elsevier 1977

Lofroth, G.; Kim, C.; Hussain, S.: Alkylating property of 2,2-dichloro vinyl dimethyl phosphate, a disregarded hazard. EMS Newsl. 2, 21-27 (1969)

Lofroth, G.: Alkylation of DNA by Dichlorvos. Naturwissenschaften 57, 393-394 (1970)

Nasta, A.; Gunther, E.: Investigations into the frequency of the chlorophyll defective plants in *Hordeum vulgare* after treating with a carbamate herbicide and combined with mutagens. (in German) Biol. Zbl. 92, 173-178 (1973)

Nilan, R.A.: The cytology and Genetics of Barley. Washington: State University Press 1964

Nilan, R.A.; Vig, B.K.: Plant test systems for detection of chemical mutagens. In: Chemical mutagens, Vol. 4 (Ed.: Hollaender, A.) New York: Plenum Press 1976

Scott, D.; Bridges, B.S.; Sobels, F.H. (eds.): Progress in Genetic Toxicology. Amsterdam: Elsevier 1977

Wild, D.: Mutagenecity testing of organophosphorus insecticides. Mutat. Res. 32, 135-150 (1975)

Wuu, K.D.; Grant, W.F.: Morphological and somatic chromosomal aberrations induced by pesticides in barley (*Hordeum vulgare*). Can. J. Genet. Cytol. 8, 481-501 (1966)

Zimmermann, F.K.: Panel discussion in sub-mammalian systems. Mutat. Res. 41, 163-170 (1976)

Received July 12, 1979
Communicated by B.R. Murty

Dr. B.B. Panda
Dr. C.B.S.R. Sharma
Cytogenetics Laboratory
Department of Botany
Berhampur University
Berhampur 760 007 (India)