Induced Somatic Aberrations and Chlorophyll Mutations in *Capsicum* by Insecticides

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Production of the chromosomal aberrations and chlorophyll mutations are essential parameters for the index of mutation frequency and for studying the biological effects (Brewen and Preston 1973, Kihlman 1975, Grant 1978, Grower and Tyagi 1979, Singh *et al.* 1980). Demonstration of mutagenicity of some common pesticides has evoked considerable interest (Epstein and Legator 1971). In recent years most of the pesticides are proved to be mutagenic agents and are causing changes in genetic constitution of organism similar to those produced by radiation. So far effect of pesticides was studied mostly on test plant system and literature on major plants is scanty. Therefore it was thought of interest to find the effects of pesticides on a commercially important vegetable crop plant like chilli.

This communication deals with the somatic chromosomal aberrations and chlorophyll mutants induced by four insecticides viz. DDVP, TARA 909, Phosphomidon and Monocrotophos on *Capsicum annum* L.

Materials and methods

Pure seeds of *Capsicum annum* L. cultivar G₃ obtained from National Seeds Corporation, Warangal, India. The seeds were soaked in distilled water for 24 hrs and then treated with insecticidal chemicals of each for 24 hr. The treated seeds were washed thoroughly for 1 hr in running tap water and sown in experimental plots along with control.

Table 1. Common or trade name of insecticide, active ingredients and source

S. no.	Trade name of the insecticide	Active ingredient	Source
1.	DDVP (97%)	2,2-Dichloroethenyl dimethyl phosphate	Hindustan Ciba-geigy Limited
2.	Monocrotophos (77.04%)	Dimethyl 1-methy -3-(Methyl-amino)-3-oxo-1-propenyl phosphate	Hindustan Ciba-geigy Limited
3.	Phosphomidon (94.7%)	2-Chloro-3-(diethylamino)-1-methyl-3-oxo-1-propenyl dimethyl phosphate	Hindustan Ciba-geigy Limited
4.	TARA 909 (30 EC) (Dimethoate)	0,0-dimethyl 3-(N-methyl carbamoylmethyl)phosphoro-dithioate.	Shaw Wallace & Company Limited

Four insecticides i.e., DDVP, TARA 909, Phosphomidon and Monocrotophos are organo-phosphorus compounds. The insecticides obtained from various commercial sources are listed in Table 1. Four concentrations, 0.1%, 0.3%, 0.5% and 1.0% of each insecticide were freshly prepared with distilled water and adjusted to a pH 7 at room temperature 27°C.

In order to study chromosomal behaviour in mitotic system, the root tips were fixed in acetic-alcohol (1: 3) and cytological preparations were made with aceto-orcein. For determi-

nation of the frequency and spectrum of chlorophyll mutations, the M_1 plants were harvested separately and sown in M_2 generation on plant progeny basis. Frequency, spectrum and segregation of various chlorophyll mutants were scored in M_2 generation.

Results

The effects of the various treatments with four insecticides on somatic chromosomes are given in Table 2. Mitotic index was severely reduced as the concentration increased. Almost all treatments induced a significant amount of cytological damage. Chromosomal aberrations were not detected in control. It was recorded that the frequency of chromosomal alterations was increasingly proportionate to the increase in concentration of chemical. Among the four insecticides DDVP induced drastic aberrations followed by TARA 909, Phosphomidon and Monocrotophos. Concerning the types of induced abnormalities disturbed meta- and

Table 2. Frequency of mitotic anomalies induced by four insecticides in *C. annuum*
L. cultivar G₃

Mutagenic agent	Treatment	Metaphase %	Anaphase %		Telophase %		Micronuclei %
		Fragments	Frag.	Brid.	Frag.	Brid.	
DDVP	0.1%	0.62	0.46	—	0.26	—	—
	0.3%	1.41	1.26	0.72	1.06	—	—
	0.5%	3.22	3.12	2.06	2.76	0.74	0.86
	1.0%	4.98	4.79	3.18	3.68	1.09	2.24
TARA 909	0.1%	0.46	0.23	—	0.19	—	—
	0.3%	1.36	0.96	0.18	0.84	—	—
	0.5%	3.09	2.83	1.96	2.58	0.69	0.74
	1.0%	4.82	4.62	2.69	3.02	0.98	1.87
Phosphomidon	0.1%	0.32	—	—	—	—	—
	0.3%	1.09	0.86	—	0.72	—	—
	0.5%	2.97	2.69	1.74	2.25	0.49	0.62
	1.0%	3.56	3.05	2.08	2.86	0.74	1.54
Monocrotophos	0.1%	—	—	—	—	—	—
	0.3%	0.91	0.74	—	0.65	—	—
	0.5%	2.28	2.09	1.14	1.87	0.34	—
	1.0%	3.22	2.97	1.78	2.26	0.51	—

anaphases comprised the most dominant type. Considerable percentage of anaphase bridges and lagging chromosomes were noticed. The common chromosomal aberrations at metaphase were unoriented chromosomes (Figs. 1, 2) and clumping of chromosomes (Fig. 3) in all treatments. At anaphase, double bridges, single bridge with fragments (Figs. 4, 7, 8) and laggards (Fig. 6) were frequently recorded. However, there were increased number of fragments present in a cell than the number of bridges. The fragments were either free or remained attached to the bridges and varied in size and shape (Figs. 5, 6). Most of the fragments were rod shape and some of them occurred in pairs (Figs. 5, 6). At telophase chromatid bridges with many fragments and chromosomal bridges with fragments were noticed (Fig. 7). Micro-nuclei presumably aggregation of fragments or laggards were also recorded (Fig. 9).

A number of chlorophyll mutant seedlings were scored in all treated samples. Spectrum of chlorophyll mutants included xantha, albina, chlorina, alboviridis, xanthoviridis and vire-scence. The data on chlorophyll mutations were scored on the basis of mutant seedlings in the total population and the data are summarised in Table 3. Chlorophyll mutation frequency

increases with increasing concentration of chemical. Different types of chlorophyll mutations observed are described below:

1. Xantha: Yellow to yellowish white, lethal (10–15 days), carotenoids present but chlorophylls absent.
2. Albina: White, lethal (6–7 days). No chlorophylls or carotenoids are formed.
3. Chlorina: Yellowish green, lethal (10–12 days, rarely 30 days). However, very few of them which were having light yellowish green survived till maturity and set a few seeds.
4. Xanthoviridis: Green with yellow apex, viable.
5. Alboviridis: Green with white apex, viable.

Figs. 1–9. 1, scattered fragments at metaphase. 2, stickiness of chromosome and fragments at metaphase. 3, pronounced stickiness at metaphase. 4, fragments at anaphase. 5, bridge and fragments at anaphase. 6, lagging chromosomes and paired fragments at anaphase. 7, single bridge and fragments at telophase. 8, double bridge, fragments and stickiness of chromosomes at telophase. 9, micronuclei at telophase.

6. Virescence: Light green, normally change into normal green viable.

It can be seen that xantha and albina types were most frequent followed by chlorina relative to others found in the M_6 generation. The present studies revealed that predominantly TARA 909 produced albina and xantha while only xantha found in DDVP and Phosphomidon treatments. Monocrotophos predominantly produced chlorina mutants. Chlorophyll mutations like those of albobiviridis, xanthoviridis and virescence recorded in M_2 generation were excluded from analysis since they were produced in a low frequency. The order of efficiency of the insecticides to induce chlorophyll mutations was as follows:

DDVP > TARA 909 > Phosphomidon > Monocrotophos

Table 3. Frequency and spectrum of chlorophyll mutations induced by four insecticides in M_2 generation of *C. annuum* L. cultivar G_3

Mutagen	Treatment	Total no. of seedlings	Chlorophyll mutants %			Total mutation %
			Albina	Xantha	Chlorina	
DDVP	0.1%	492	—	1.01	0.56	1.57
	0.3%	469	0.67	1.69	0.98	3.34
	0.5%	408	2.11	3.88	1.24	7.23
	1.0%	386	3.45	4.31	2.09	9.85
TARA 909	0.1%	529	0.91	0.48	0.28	1.67
	0.3%	496	1.47	0.97	—	2.44
	0.5%	468	2.68	1.44	1.03	5.15
	1.0%	406	3.94	2.56	1.36	7.86
Phosphomidon	0.1%	545	0.21	0.56	0.27	1.04
	0.3%	452	0.68	1.08	0.84	2.60
	0.5%	422	1.36	2.46	1.05	4.87
	1.0%	396	2.34	3.40	1.38	7.12
Monocrotophos	0.1%	548	—	0.37	0.49	0.86
	0.3%	479	0.31	0.48	1.08	1.87
	0.5%	448	0.72	0.91	2.09	3.72
	1.0%	402	1.13	1.67	3.22	6.02

Discussion

The four insecticides used presently exert a marked mitodepressive action and induced chromosomal aberrations in *C. annuum* root tip cells. It is obvious that frequency of aberrations depend on concentration. Mitotic activity reduced and frequency of aberrations increased as the concentration increased. It is worth mentioning that other pesticides such as Diamine, Simazine and Linuron (Shehab 1971), Rogor IPC (Amer and Farah 1974, 1975), Leptophos (Amer and Farah 1979), Carbamates (Tomkins and Grant 1972, Tobgy *et al.* 1969, Wu and Grant 1966, Badr 1983a, b, Badr and Elkington 1982) also showed similar results.

The insecticides produced mitotic and a mitotic effects. Mitotic abnormalities are generally regarded as the result of an action on the spindle apparatus, whereas chromotoxic effects indicate effects on chromosomes either directly or indirectly. All insecticides produced cytogenetic effects such as bridge formation, micronuclei and to some extent premature chromosome condensation. The first two may lead to loss of genetic material and the third suggests direct action of chemical on genetic material (Njagi and Gopalan 1981). This was reported earlier by Badr (1983a, b). All insecticides induced chromosomal stickiness, breaks and bridges. Stickiness is prominent, it is generally regarded as physiological and unspecific disturbance and has been attributed to an action on the proteins of chromosomes, similar results were observed by El-Sadek (1972) and Tomkins and Grant (1972). The breaks and laggards

may be due to a major interaction of the insecticides in the cells may be with the proteins of the spindle apparatus and the proteins which form an integral part of the chromosomes (Wolff 1963, Wu and Grant 1966). Since enzymes are known to play a major role in maintaining the numerous biochemical processes which occur in a cell, the application of pesticide may destroy, inhibit or enhance the synthesis of certain enzymes and so upset the cell system (Wu and Grant 1966). The stickiness, breaks and bridges were reported with other pesticides (Tobgy *et al.* 1969, Amer and Farah 1976, Badr 1983a, b and Njagi and Gopalan 1981).

The chromosomal bridges may be attributed to the general stickiness of chromosomes and the subsequent failure of anaphase separation (Abraham and Koshy 1979) or it may be the result of chromosome breakage and reunion (Tomkins and Grant 1972, Grant 1978, Badr 1983a, b). Presence of paired fragments at anaphase and telophase in the present observations indicates monopartite chromosome behaviour and this was also supported by Kalloo (1972) in *Pisum*. Some lagging chromosomes may have gradually clumped to form micronuclei; micronuclei are true mutagenic effects (Auerbach 1962).

In higher plants no study appears to have been made to estimate the chlorophyll mutants induced by pesticides. Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments. The results of this experiment revealed the genotypic differences in response were observed in the form of frequencies of induced chlorophyll mutations in the M_2 generation. The same had been reported earlier in *Hordeum vulgare* (Wu and Grant 1966, Grower and Tyagi 1979, Singh *et al.* 1980). Wheat (Goud 1967), Mungbean (Rathnaswamy *et al.* 1978, Bahl and Gupta 1982), with *Pisum* (Lofroth *et al.* 1969), Mohandas and Grant (1972) reported albina mutant with 2,4-D.

The ability of these insecticides to cause chlorophyll mutations may be due to their DNA alkylating properties. However, the variations in the mutation frequencies may be a function of the relative efficiencies of the insecticides to reach and/or act on the cellular target (Panda and Sharma 1979). It is generally believed that ionizing radiation produced a high frequency of albinas whereas the chemical mutagens produced high frequency of other types (Gustafsson 1963). Contrary to above, the results revealed the xantha and albina were the most frequent types. The frequency of chlorophyll mutations was found to be concomitant with concentration of treatment. Dose dependent increase in the mutation frequency has been reported by several workers in different plant systems (Prasad and Das 1980, Sadanandam *et al.* 1981, Venkatrajam *et al.* 1984).

The present observations reveal that the insecticides are potentially capable of producing a variety of mutations and chromosomal aberrations in chilli and are potent mutagens as other established mutagens with radiomimetic action.

Summary

Mutagenic effects of four insecticides on soaked seeds of *Capsicum annum* cultivar G_3 have been studied. The insecticides used presently induced various types of chromosomal alterations in meristematic cells. Details of aberrations are described and probable reasons for their induction are discussed. Chlorophyll mutations of six types, viz., xantha, albina, chlorina, xanthoviridis, alboviridis and virescence were recorded in M_2 generation. The present findings reveal that four insecticides potentially are capable of producing a variety of mutations in chilli and are potent mutagenic agents with radiomimetic action.

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