

Cytogenetic Effect of Sulphur Dioxide on
Vicia faba plant.

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Introduction

Atmospheric emissions of SO₂ are a major pollution problem, that has been continually growing due to the increasing use of fossil fuels "coal and tar" to generate electricity.

Regulations have been passed in many countries to set legal limits to SO₂ emissions. High levels were permitted within the industrial workplaces. Stokinger (1972) mentioned that, 5 ppm SO₂, is the justified industrial limit of SO₂ emission.

Compliance has been made difficult, due to, the relatively short supply of "clean" low sulphur fuels and the cost of the alternative methods of SO₂ control (Collins 1971, Dunham et al., 1974 and Abelson 1975).

In Egypt, SO₂ levels are very high when compared with other parts of the world. In the polluted Abu Zaab area (40 km north east Cairo), it ranged from 23 to 120 to 5 ppm at 50, 500 and 1000 meters away respectively (Kord 1981).

Kord (1981) found that, treating different plants with SO₂ (5, 10 and 25 ppm) caused retardation of growth. She found also that, fumigation of Raphanus sativus and Eruca sativa with 5 and 10 ppm sulphur dioxide (³⁵S-labelled sodium sulphite, specific activity = 53 mCi/g) for 30 and 60 minutes, increased the sulphur content of the fumigated plants.

Fumigation of Vicia faba plants with 5 ppm SO₂ for a period of 5 hours caused the induction of a highly significant percentage of abnormal PMC's/plant. Such effect increased steadily with the increase of the gas concentration and the period of fumigation (Amer et al. 1987-I).

In the present investigation, the cytogenetic effect of SO₂ after fumigation of Vicia faba plants with different concentrations of the gas (5 - 30 ppm) for 4 hrs /day X 4 days has been studied on the meiosis and pollen viability. Such effect has been followed in groups of treated plants which were left to recover for 6 and 12 days.

Materials and Methods

Vicia faba (V. Giza 2) plants, were fumigated for 4 hrs/day X 4 successive days with different concentrations of SO₂ (5 - 60 ppm). Compare Amer et al. (1987-I) for the detailed method.

A group of the treated plants were left to recover for 6 and 12 days.

Results

Pollen mother cells :

Fumigation of Vicia faba plants with 5 ppm SO₂ 4 hrs/day X 4 days affected the induction of 26 % abnormal PMC's/plant. Such percentage increased to a great extent as the concentration of the gas was increased. A high depression in the percentage of abnormal PMC's/plant occurred when the treated plants were left to recover for 6 and 12 days (Fig. 1).

The highest percentage of the induced abnormalities was observed in the anaphase stages. The percentage of abnormal PMC's in meiotic division II was higher than that in meiotic division I (Table I).

Table (II) represents, the different types of abnormalities observed in the PNCs of the plants fumigated with SO_2 and those which were left to recover for 6 and 12 days.

Tetrads :

The percentage of abnormal tetrads was 8.6 % after fumigation with 5 ppm SO_2 , and reached 61 % after fumigation with 30 ppm of the gas. Such percentage decreased to a great extent after recovery for 6 and 12 days (Table I).

The presence of more than tetra-group of cells (pentads, hexads and heptads) and dyads (e.g. Fig. 2) instead of the usual tetrads dominated. Its percentage reached 20 % and 27 % of the scored tetrads respectively after fumigation of the plants with 30 ppm SO_2 . Tryads were observed in a lower frequency, its percentage did not exceed 6 % of the scored tetrads after fumigation with the different concentrations of SO_2 . Tetraploid monads (Syncytes e.g. Fig. 2) were observed (4 %) only after fumigation of Vicia faba plants with 30 ppm of the gas.

Pollen grains :

The percentage of abnormal PGs was generally, much lower than that of abnormal tetrads after fumigation of Vicia faba plants with the different concentrations of SO₂ (Table I).

The nonviable PGs and the small ones were the dominating types of abnormalities observed after treatment with the different concentrations of the gas. The percentage of the small PGs reached 4 % of the scored PGs after fumigation of the plants with 30 ppm SO₂. Tetraploid pollen monads (Syncyte pollen formations e.g. Fig. 4), pollen dyads (e.g. Fig. 3) and tryads as well as deformed PGs were also observed, but, in a lower frequency.

Discussion

Repeated fumigation (4 hrs/day X 4 days) with the different concentrations of SO₂ (5 - 30 ppm) affected the induction of a high percentage of abnormal PMCs/Viola faba plant. Rapid was the increase in this percentage with the increase of the gas concentration. Almost all the PMCs were abnormal after fumigation with 30 ppm SO₂, and some dead PMCs were observed in

the flower buds of the plants fumigated with this concentration of SO_2 . Thus, a dose about 20 ppm can be considered as the most tolerable concentration for 4 hrs/day X 4 days fumigation of Vicia faba plants with SO_2 .

The percentage of abnormal tetrads was high, especially, after fumigation of the plants with the higher concentrations of SO_2 , and was proportional to the gas concentration.

More than tetra-group of cells was the dominant type of abnormalities observed in this stage after fumigation with 5 ppm SO_2 . Such aggregates of cells may result from multipolar telophase II cells as Amer and Farah (1976) suggested in their study on the effect of the carbamate pesticides "IPC", "Rogor" and "Duphar" on Vicia faba plants.

The diploid dyads, unequal dyads and tryads, seemed to be formed as a result of the observed irregular distribution of the chromosomes in the different meiotic stages.

Small pollen grains seemed to be formed as a result of the induction of multipolar or unequal distribution of the chromosomes. Syncyte pollen formation seemed to be formed as a result of the absence cytokinesis.

Sudharshan and Jagadishchandra (1981) reported on the occurrence of synocyte formation and other meiotic irregularities during microsporogenesis in a wild population of Cymbopogon caesius (Poaceae). Montezuma*De,Carvalho (1973) in his study on the effect of nitrous oxide (N_2O) on meiosis of Tradescantia paludosa reported that, nitrous oxide under pressure inhibited the meiotic spindle and gave rise to restitution nuclei (C-interphase). When such cells were allowed to recover, they entered in the 2nd division of meiosis and the spindle was reformed again. Such cells would form diploid pollen dyads.

It is worthy to mention that, a much higher percentage of abnormal : PMCs; tetrads and nonviable PGs was observed in the plants fumigated with SO_2 for 4 hrs/day X 4 days than that observed in the plants fumigated with SO_2 for 5 hrs (Amer et al. 1987-I). Such effect may be attributed to the greater ability to take up SO_2 displayed by young leaves (Surrounding the flower buds) than mature or senescent leaves (Guderian 1970).

Vicia faba plants fumigated with SO_2 did not recover after 12 days. A statistically significant percentage of abnormal PMCs and nonviable PGs/plant was still observed, even, in the plants fumigated with the lowest concentration of SO_2 (5 ppm). In this concern it is worthy to,

mention that, after daily exposure of the grossly sulphite oxidase-deficient rats to 10 and 30 ppm SO₂ for 6 h/day for 99 days, the testes of the rats were severely atrophied and devoid of spermatogenic cells. There was no evidence for recovery 4 wk following the termination of exposure (Gunnison et al. 1987).

Taking chromosome fragmentation as the criterion for mutagenic potential, it was found that, the percentage of PNCs with fragments increased progressively with the increase of SO₂ concentration (Fig. 5).

It is evident from this study that, repeating exposure to different concentrations of the common air pollutant SO₂ caused adverse genetic hazards for Vicia faba plants. The gas may cause hazardous effects for other cultivated plants. So, arise the need, for more developed not expensive methods for SO₂ emission control, for the protection of the genetic information from a mutagen contaminating the biosphere.

Summary

In the present investigation, the cytogenetic effect of fumigation of Vicia faba plants, for 4 hrs/day X 4 days with different concentrations of SO₂ (5-30 ppm), has been

studied on the meiosis and pollen viability. Such effect has been followed in groups of treated plants which were left to recover for 6 and 12 days.

Repeated fumigation (4 hrs/day X 4 days) with the different concentrations of SO_2 (5 - 30 ppm) affected the induction of a high percentage of abnormal PMCs/Vicia faba plant. The highest percentage of the induced abnormalities was observed in the anaphase stages. The percentage of abnormal PMCs in meiotic division II was higher than that in meiotic division I.

The percentage of abnormal tetrads was high, especially after fumigation of the plants with the higher concentrations of SO_2 . The presence of more than tetragroup of cells (pentads, hexads and heptads) and dyads instead of the usual tetrads dominated.

The percentage of abnormal PGs was generally, much lower than that of abnormal tetrads after fumigation of the plants with the different concentrations of SO_2 . The nonviable PGs and the small ones dominated.

Vicia faba plants fumigated with 5-30 ppm SO_2 for 4 hrs/day X 4 days did not recover after 12 days. A statistically significant percentage of abnormal PMCs

and nonviable PGs/plant was still observed, even in the plants fumigated with the lowest concentration (5 ppm).

Taking chromosome fragmentation as the criterion for mutagenic potential, it was found that the percentage of PMCs with fragments increased progressively with the increase of SO₂ concentration.

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Table I : Percentage of abnormal PMCS, tetrads and nonviable Pgs/plant, after fumigation with different concentrations of SO₂ for 4 hrs/day X 4 days and recovery for a period of 6 and 12 days.

Conc. Period (ppm) of recovery	Scored No.	PMCS		Scored No.	% abn.	Tetrads		Scored No.	% viable	Pgs	
		1st div.	% abn.			2nd div.	% abn.			Mean % ± S.E.	Abn. Pgs nonviable
30 -	3665	89.51	93.02	1500	60.53	18000	11.66	6.917 ± 0.820 _{ME}			
6 days	3595	25.26	33.14	1800	8.33	6000	3.09	3.583 ± 0.300 _{ME}			
12 days	4345	10.40	14.02	2400	2.29	6000	1.43	1.400 ± 0.245 _{ME}			
20 -	7290	59.90	71.10	3100	40.29	12000	7.29	4.383 ± 0.520 _{ME}			
6 days	4370	16.71	22.78	2400	3.75	6000	1.24	2.333 ± 0.247 _{ME}			
12 days	5750	6.45	9.26	2400	1.67	6000	0.69	0.983 ± 0.079 _{ME}			
10 -	5350	36.39	42.88	3460	21.67	12000	4.47	1.883 ± 0.260 _{ME}			
6 days	6050	9.20	16.61	2400	1.67	6000	0.77	0.900 ± 0.155 _{ME}			
12 days	5355	4.03	5.13	2400	0.71	6000	0.25	0.417 ± 0.060 _{ME}			
5 -	4932	26.96	26.25	2250	8.58	6000	1.70	0.683 ± 0.100 _{ME}			
6 days	5800	5.56	9.44	2400	0.33	6000	0.35	0.366 ± 0.049 _{ME}			
12 days	5260	2.15	2.31	2400	0.08	6000	0.17	0.133 ± 0.033 _{ME}			
Cont. -	4390	1.17	2.86	2400	1.04	6000	0.57	0.100 ± 0.040			
6 days	4580	0.66	1.30	2400	0.08	6000	0.07	0.083 ± 0.017			
12 days	4040	0.42	0.65	2400	-	6000	0.02	0.050 ± 0.022			

* Significant from control at 0.01 level (t-test).

Table II: Number and percentage of the occurring abnormalities in the melons of *Vitola febe* plants, after fumigation with different concentrations of SO₂ for 4 hrs/day x 4 days and recovery for a period of 6 and 12 days.

Period of recovery	Conc. (ppm)	No. abn. PNCs	Relative to the number of abnormal PNCs											% of PNCs with multiple type of abn.	
			Stick.	Bird.	Deap.	Prags.	Bird. with Irreg.	Dist.	Retard. sep.	Unequal dist.	Uncl. pol.	Retra- ploid.	Leg. nu.		
6 days	30	3363	16.09	10.35	4.67	15.79	5.53	35.56	0.56	2.85	9.76	0.09	17.54	0.18	15.97
	20	4959	7.06	12.16	1.65	15.11	2.44	29.54	0.54	5.71	15.94	0.24	24.54	0.48	13.79
	10	2109	18.07	10.34	0.33	18.40	2.28	12.00	0.66	7.35	16.83	-	29.30	0.96	14.53
	5	1310	8.18	18.86	-	17.41	3.45	8.14	0.95	2.97	18.56	-	26.13	0.87	5.66
	30	1046	8.90	18.08	2.58	9.56	11.17	11.86	0.76	1.82	24.75	-	19.49	-	8.26
12 days	30	533	14.07	27.39	-	7.51	4.32	7.69	-	0.75	25.33	-	20.26	-	6.54
	20	455	13.41	24.18	-	4.62	7.69	8.79	-	2.42	22.20	-	21.98	-	4.76
	10	240	14.58	23.75	-	5.00	4.17	5.42	-	2.92	28.33	-	28.33	-	11.50
	5	117	5.98	34.19	-	7.69	4.27	1.71	-	-	17.09	-	35.90	-	6.45
	30	533	14.07	27.39	-	7.51	4.32	7.69	-	0.75	25.33	-	20.26	-	6.54

* The sum of the percentages of the different types of abnormalities is more than 100, because the PNCs which have more than one type of abnormalities was recorded under those types in the same time.

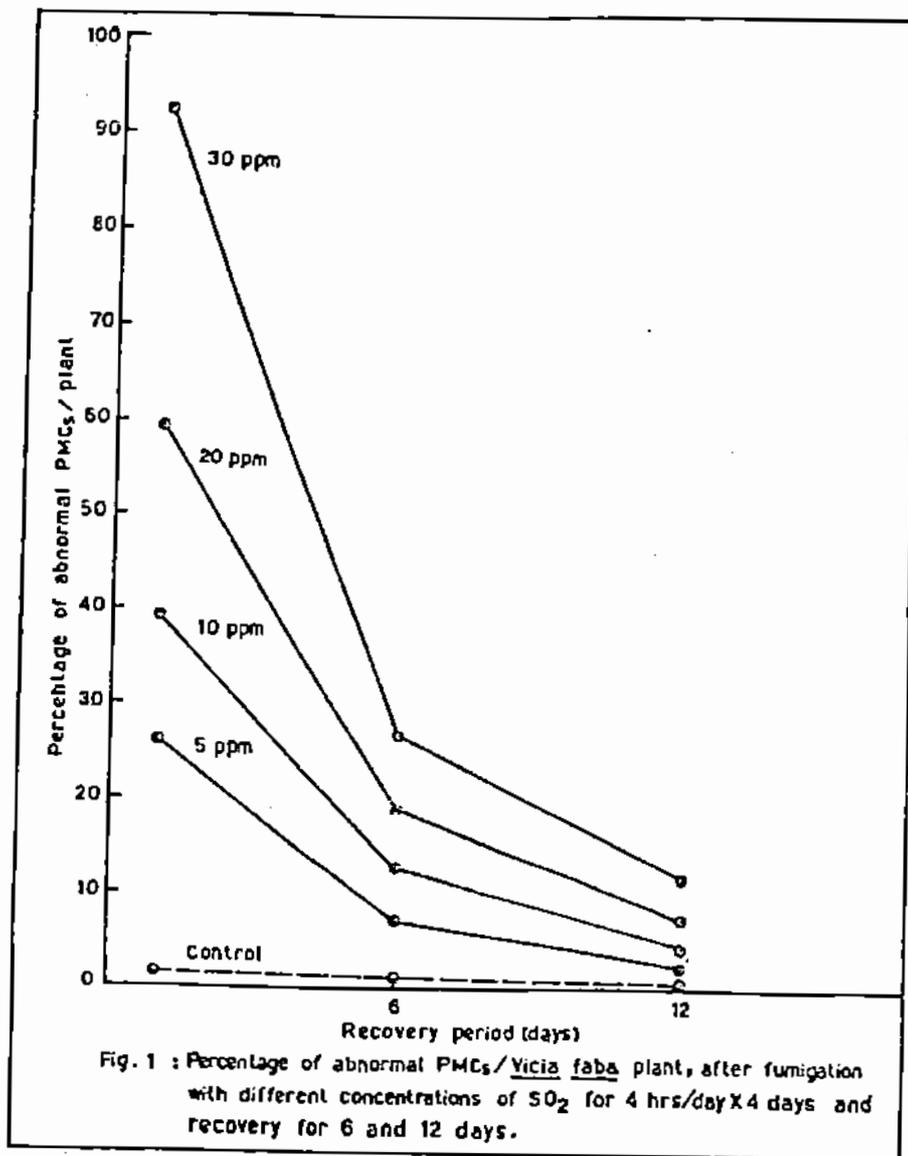




Fig. 2



Fig. 3



Fig. 4

Figs 2-4 : Dyad consisting of two equal cells (left) and other consisting of two unequal cells (right) and tetraploid monad (Syncyte formation) below (Fig. 2) pollen dyad (Fig. 3) and tetraploid pollen monad (Syncyte pollen formation, Fig.4), after fumigation of Vicia faba plants with 10 ppm (Fig. 2) and 30 ppm (Figs. 3 and 4) SO₂ for 4 hrs/day X 4 days.

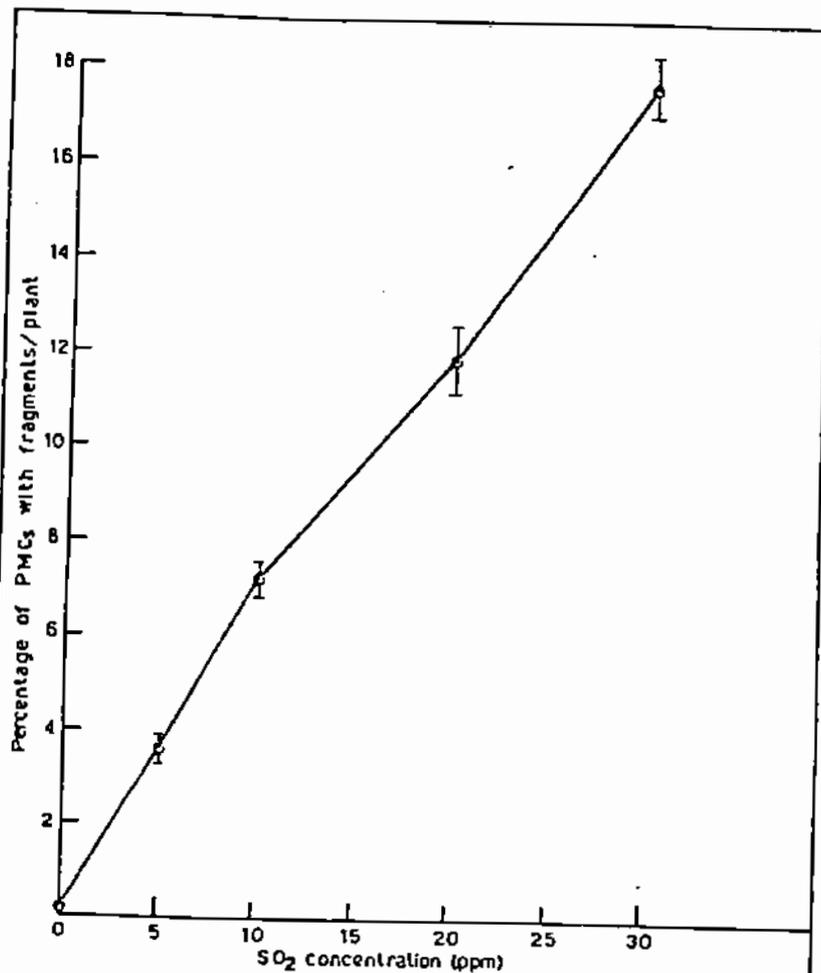


Fig. 5 : Percentage of the abnormal PMCs with fragments/*Vicia faba* plant, after fumigation with different concentrations of SO₂ for 4 hrs /day X 4 days.

Legends

Fig. 1 : Percentage of abnormal PMCs/Vicia faba plant after fumigation for 4 hrs/day X 4 days with different concentrations of SO₂ and recovery for 6 and 12 days.

Figs.2-4: Dyad consisting of two equal cells (left) and other consisting of two unequal cells (right) and tetraploid monad (Syncyte formation) below (Fig. 2) pollen dyad (Fig. 3) and tetraploid pollen monad (Syncyte pollen formation, Fig. 4); after fumigation of Vicia faba plants with:10 ppm (Fig. 2) and 30 ppm (Figs. 3 and 4) SO₂ for 4 hrs/day X 4 days.

Fig. 5 : Percentage of the abnormal PMCs with fragments/Vicia faba plant, after fumigation for 4 hrs/day X 4 days with different concentrations of SO₂.