Chromosomal Abnormalities Arising Under The Action Of Antibiotics In *Pisum Sativum*

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Abstract

Studies on genetic as well as cytological effects of antibiotics in higher plants are scanty at present. Therefore, in this study we tried to investigate chromosomal and gene mutations arising under the action of antibiotics. *Pisum sativum* (2n=14) which belongs to sub family *papilionaceae* of family *leguminosae* and has comparatively small number of good size chromosome and is a very important legume crop of India, has been chosen as test system for evaluating mutagenic potency of some of the antibiotics. The effect of antibiotics (viz., amoxicillin, streptomycin and tetracycline) treatment on biological damage and genetic changes in M1 and subsequent generation (M2) of its progeny were evaluated in relation to the following parameters; germination, seedling injury, cytological observations, pollen sterility. [Nature and Science. 2009;7(3):104-112]. (ISSN: 1545-0740).

Introduction

Antibiotics are the substances which are produced by microorganism and act against microorganism (e.g., *Pencillium notatum*). Most antibiotics known till date are products of actinomycetes and some are from fungi and bacteria (e.g., *Streptomyces* spp.). Most antibiotics have been tried for plant disease control. The commonly used antibiotics are streptomycin, tetracycline, griseofulvin, cycloheximide and aurofungin. Although antibiotics are used for disease control, it has many side effects, one of them being gene mutations. These antibiotics enter in the tissues/organs as chemical compounds and penetrate membrane system. Chemical reaction may occur before a pharmacodynamic action and metabolites formed may cause unwanted toxic effects before being excreted. If these chemical compounds react with genetic material (DNA) heritable changes (beneficial/harmful) may be induced. EMS (Svetleva *et. al*., 2005), DES and some antibiotics are being used extensively for inducing mutation in the microbes, lower plants (Takano *et. al*., 2003) and higher plants. Most of the antibiotics affect DNA, RNA and protein synthesis. Antibiotics induce chromosomal abnormalities such as erosions, diplo chromatids, pycnosis, micronuclei, bridges with or without fragments etc. Induction of genetic damage by mitomycin-c has also been reported (Vig, 1997). Mutagenic potency of antibiotic has been demonstrated in *Phaseolus vulgaris* L (Prasad *et. al*., 1981). All these observations necessitate the screening of antibiotics for the harmful short term and overall insidious long term effect on human health and of noxious effects on plants, bacteria and animals. The mutagenic action of antibiotics as well as chemicals has been extensively evaluated with various tests on plants like wheat, barley (Ehrenberg, 1971), maize (Nilan *et. al*., 1976), tradescentia (Taro, 1982), soybean (Carroll *et. al*., 1985), but *Pisum sativum* (Duc, 1989) being one of the pillars of the classical genetics has not been commonly used in mutagenic studies. *Pisum sativum* L (2n=14) has 7 pairs of recognizable chromosomes (3 large, 3 medium and 1 small chromosome) out of which 3 pairs are metacentric, 4 are submetacentric or subterminal. The average size of the chromosomes falls within the range of 4.29µ to 7.12µ.

Materials and Methods

Seeds (variety ps-49) were obtained from seed production center of G.B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand).

Seed treatment:

Dry and dormant seeds of *P. sativum* were treated separately with 0.1, 0.2, 0.3, 0.4 and 0.5% freshly prepared solutions of amoxicillin, streptomycin and tetracycline for 4 hrs with constant shaking under laboratory conditions (24±2°c). Control seeds were also soaked in distilled water for the same period for which treatment was given. All treated seeds were thoroughly washed to remove the trace of

chemicals. There after 50% of seeds in single layer were allowed to germinate on moist filter paper and 50% were directly sown in the field.

Field preparation:

Land was prepared by using well decayed cow dung manure. Total 100 seeds for each dose were sown in 4 different plots of size 2.25 m^2 each with row to row distance of 30 cm, plant to plant distance 30 cm and 25 seeds were sown per plot. Control seeds were sown adjacent to the treated seeds in the same manner as that of treated seeds. M_1 seeds were harvested plant wise and were sown directly in the next season to raise M_2 progenies. M2 seeds were also collected plant wise. Different observations were made for both M1 and M2 generations. Data collected for different characters in M1 and M2 generations were statistically analyzed.

Results

Germination:

Germinated seeds of both control as well as treated were counted every 24 hrs from the time of treatment till 10^{th} day. Total number of seeds germinated till 10^{th} day after treatment were used to study the effects of antibiotics on germination, both in M1 and M2 generations.

It was observed that tetracycline is more toxic than amoxicillin and streptomycin with regards to effect on percent germination. The reduction in germination frequency at its lowest dose 0.1% (53.3% that of control) is much lower than the percent germination seen at the highest concentration that is 0.5% of either amoxicillin (81.35% that of control) or streptomycin (83% that of control) (Table 1).

Seedling injury:

Ten germinated treated and untreated seeds were randomly selected for measurement of root and shoot length. The root and shoot length were measured every 24 hrs after drawing their outline on centimeter graph paper. The lengths of root and shoot were read from the graph paper every day up to 10^{th} day, from the day of emergence of root and shoot. Data on mean root and shoot length on 10^{th} day after their emergence, at M1 and M2 are summarized in Table 2 and 3, respectively.

A dose dependent decrease in both root and shoot length in M1 and M2 was noticed, although both root and shoot length at M2 were found to be longer than M1 at all concentrations of antibiotics. Tetracycline was found to be most effective, exhibiting 53% and 78% reduction in root and shoot length, respectively, at 5% concentration, where as, the corresponding reductions were 34% and 39% in streptomycin treatment and 28% and 31%, respectively, in case of amoxicillin treatment.

Correlation coefficient between root and shoot length at M1 generation for amoxicillin, streptomycin and tetracycline and at M2 for amoxicillin and streptomycin indicates highly significant ($p>0.001$) positive co-relation between root and shoot length for both amoxicillin and streptomycin, and significant negative co-relation for shoot length in case of tetracycline treatment in M1 generation. However, positive correlation at lower doses, 0.1-0.2% of amoxicillin and 0.1-0.3% and 0.4-0.5% streptomycin was observed in M2 generation. The results further indicate that shoot is more sensitive to antibiotics treatment than root (Table 4).

Cytological observations:

Root tip mitosis and PMCs were studied at M1 and M2 generations. Chromosomal abnormalities were scored at different phases of both mitosis and meiosis and chromosomal clumping was the most common effect observed at all concentration of all antibiotics. Maximum number of such type of chromosomes (28.35%) was recorded in tetracycline treatment at M1 generation (Table 7). Occurrence of micronuclei was observed only at higher concentrations of both amoxicillin and streptomycin (Table 5, 6). It was evident from the data that the frequency of a particular type of dose and the nature of chromosomal aberration is both under the influence of dose and chemical composition of antibiotic.

Similarly, in case of meiosis, chromosomal abnormalities were observed at different stages of meiosis until the formation of pollen grain. All the treated chromosomes showed bridges, fragments, laggards and bridges with or without fragments. The frequency of occurrence of such abnormalities increased with the increasing concentration of the antibiotic.

Pollen sterility:

One of the commonest features of mutagenic treatment is pollen sterility which is associated with the induction of chromosomal changes involving translocation, inversion and deletion. Higher numbers of sterile pollens were observed after amoxicillin treatment as compared to streptomycin

treatment in both the generations. Comparing the mean and variance in M1 and M2 generations, four different relationship have been discerned and are listed below (a) increased mean and decreased variance (0.5% amoxicillin at M1, 0.1% at M2, 0.5% streptomycin at M1 and 0.1% streptomycin at M2) (b) decreased mean and decreased variance (0.1% amoxicillin and 0.1% streptomycin at M2) (c) decreased mean with increased variance (0.5% amoxicillin at M1 and 0.1% amoxicillin at M2, 0.1% and 0.4% streptomycin at M2) (d) increased mean and equal variance (0.5% amoxicillin and streptomycin at M1 and 0.4% streptomycin at M1 and M2). Increased CV (%) then control except 0.1% amoxicillin at M2 was noticed at almost all the concentration of amoxicillin and streptomycin.

Table 1: Effect of antibiotics on the 10th day of germination following (% that of control) treatment

Treatment (Percent)	Generation	Amoxicillin	Streptomycin	Tetracycline
0	M1	100.00	100.00	100.00
	M2	100.00	100.00	100.00
0.1	M1	94.00	96.00	53.30
	M2	89.33	94.66	0.00
0.2	M1	90.66	93.33	50.60
	M2	82.66	88.00	0.00
0.3	M1	88.00	90.00	46.60
	M2	74.66	83.00	0.00
0.4	M1	86.60	85.00	45.30
	M2	70.66	80.00	0.00
0.5	M1	81.30	83.00	40.00
	M2	70.00	78.00	0.00

Table 2: Effect of antibiotics on mean length (cm) of roots and shoots on 10th day of emergence on M_1 generations

Treatment (Percent)	Root/Shoot	Amoxicillin	Streptomycin	Tetracycline
		Mean±S.E.	Mean±S.E.	Mean±S.E.
Control	R	6.42±0.168	6.42±0.168	6.42±0.168
	S	10.4±0.724	10.4±0.724	10.4±0.724
0.1	R	5.86±0.437	5.0±0.160	3.06±0.248
	S	9.10±0.367	9..1±0.303	2.12±6.231
0.2	R	5.54±0.36	5.18±0.147	2.72±0.258
	S	9.0±0.416	8.3±0.520	1.34±0.180
0.3	R	5.50±0.063	4.92±0.120	2.64±0.309
	S	8.74±0.223	7.66±0.460	1.24±0.121
0.4	R	4.16±0.153	4.88±0.245	2.60±0.459
	S	7.72±0.708	6.98±0.75	1.28±0.263
0.5	R	4.28±0.306	4.56±0.30	2.52±0.314
	S	7.2±0.599	6.4±0.92	1.18±0.174

Table 3: Effect of antibiotics on mean length (cm) of roots and shoots on 10th day of emergence on M2 generations

Treatment (Percent)	Root/Shoot	Amoxicillin	Streptomycin
		Mean±S.E.	Mean±S.E.
Control	R	10.74±0.480	10.74±0.480
	S	14.26±0.180	14.26±0.180
0.1	R	9.1±0.303	8.42±0.260
	S	10.12±0.591	13.14±0.291
0.2	R	7.58±0.222	8.20±0.268
	S	9.30±0.258	11.64±0.098
0.3	R	7.12±0.290	7.46±0.447
	S	8.94±0.401	10.8±0.243
0.4	R	7.16±0.476	7.18±0.136
	S	8.2±0.260	10.3±0.243
0.5	R	6.98±0.359	7.04±0.093
	S	7.72±0.708	9.0±0.310

Table 4: Correlation coefficient between root and shoot following treatment with antibiotics at M1 and M2 generation

Treatment (Percent)	Generation	Amoxicillin	Streptomycin	Tetracycline
Control	M1	0.360***	0.360***	0.360***
	M2	0.291**	0.291**	0.291**
0.1	M1	0.382***	0.714***	–0.0500***
	M2	0.412***	0.006	0.00
0.2	M1	0.188	0.332***	–0.0551
	M2	0.686***	0.780***	0.00
0.3	M1	0.825***	0.458***	0.285**
	M2	–1.00	0.086	0.00
0.4	M1	0.583***	0.451***	–0.246*
	M2	0.165	0.507***	0.00

0.5	M1	0.746***	0.447***	–0.263**
	M2	0.398***	–0.036	0.000

***Significant at 0.1% level
**Significant at 1% level
*Significant at 5% level

Table 5: Chromosomal abnormalities induced by different doses of amoxicillin in *Pisum sativum* L.

Treatment (Percent)	Generation	Total number of cells scored	Total number of dividing cells	Metaphase		Anaphase			Telophase	
				Fragment (%)	Clumped cells (%)	Bridge (%)	Bridge with F (%)	Erosion (%)	Bridge (%)	Micro nuclei (%)
0.1	M1	2576	193	–	6.40	4.48	–	–	–	–
	M2	2511	254	–	4.00	–	–	–	–	–
0.2	M1	2264	134	–	8.17	5.61	3.00	–	–	2.45
	M2	2060	190	–	7.52	1.05	2.00	–	–	1.29
0.3	M1	2564	145	5.44	8.98	5.75	2.34	2.93	–	3.52
	M2	2096	179	–	6.58	2.15	2.00	–	–	2.00
0.4	M1	2694	139	7.61	10.51	6.07	3.28	2.18	1.0	4.78
	M2	2664	165	1.26	3.24	3.29	1.77	–	–	2.56
0.5	M1	2500	124	9.76	11.01	10.41	4.18	1.5	1.5	6.56
	M2	2550	146	2.74	4.29	2.70	2.45	–	–	3.98

Table 6: Chromosomal abnormalities induced by different doses of streptomycin at M_1 and their progenies M_2

Treatment (Percent)	Generation	Total number of cells scored	Total number of dividing cells	Metaphase		Anaphase			Telophase	
				Fragment (%)	Clumped cells (%)	Bridge (%)	Bridge with F (%)	Erosion (%)	Bridge (%)	Micro nuclei (%)
0.1	M_1	2615	216	–	7.42	5.99	2.91	–	–	–
	M_2	2264	232	–	3.52	–	–	–	–	–
0.2	M_1	2540	194	5.3	8.56	7.03	4.96	1.0	–	3.0
	M_2	2079	164	2.3	3.96	2.2	–	–	–	2.0
0.3	M_1	2028	140	6.46	8.95	7.33	4.99	2.0	2.4	5.95
	M_2	2138	185	3.96	5.30	3.0	1.0	–	–	2.0
0.4	M_1	2928	169	8.98	11.56	7.44	5.0	2.76	2.0	6.29
	M_2	2305	173	4.20	6.50	3.56	2.5	2.4	–	3.28
0.5	M_1	2324	122	9.0	13.00	8.62	5.5	1.92	3.4	6.56
	M_2	2756	175	5.2	6.95	4.0	3.8	1.0	–	3.98

Table 7: Chromosomal abnormalities induced by different doses of tetracycline at M1 generation

Treatment (Percent)	Total number of cells scored	Total number of dividing cells	Metaphase % clumped cells
0.1	2984	165	11.2
0.2	2806	139	18.15
0.3	2629	112	20.85
0.4	2820	135	25.76
0.5	2694	112	28.35

Table 8: Effect of Amoxicillin on pollen sterility in *Pisum sativum* at M1 and their progeny M2

Treatment (Percent)	Generation	Number of pollen grains scored	Sterility (%) ± S.E.*	Variance	C.V. (%)
Control	M1	722	8.03±1.20	7.29	23.27
	M2	692	7.28±1.09	5.99	18.84
0.1	M1	898	13.47±3.0	45.19	27.76
	M2	596	8.53±0.368	0.676	8.48
0.2	M1	762	16.92±4.66	108.69	40.38
	M2	659	9.76±2.19	24.05	38.07
0.3	M1	694	19.74±4.13	85.29	33.68
	M2	589	10.37±2.36	27.87	43.12
0.4	M1	629	23.52±4.70	110.79	35.54
	M2	856	12.61±3.07	47.29	31.80
0.5	M1	598	26.42±5.81	169.29	41.17
	M2	739	15.96±3.38	57.29	32.0

(* S.E. of mean)

Table 9: Effect of streptomycin on pollen sterility in *Pisum sativum* at M1 and their progeny M2

Treatment (Percent)	Generation	Number of Pollen grains scored	Sterility (%) ± S.E.*	Variance	C.V. (%)
0.1	M1	728	15.65±2.72	37.19	26.71
	M2	538	11.15±1.84	16.99	34.34
0.2	M1	694	18.87±4.75	113.19	40.57
	M2	738	13.77±4.40	96.79	47.75
0.3	M1	605	25.28±4.76	113.29	34.77
	M2	698	17.19±3.98	79.49	37.12
0.4	M1	525	28.0±5.42	147.29	41.25
	M2	629	23.05±5.44	148.49	42.0
0.5	M1	495	31.51±4.50	101.69	32.30
	M2	548	25.36±3.96	78.69	31.90

(* S.E. of mean)

Discussion

The reduction in germination percentage observed in the present case may be due to any one of the following effects of antibiotic treatment (a) disruption of mechanism of gene action controlling protein synthesis (b) inhibition of several enzyme systems responsible for germination (c) chromosomal damages (Sinha *et. al*., 1972) (e) dissimilar mode of its action. Growth depression had been also affected in almost equal proportion in amoxicillin and streptomycin treatment. However, the shoot depression was relatively very much pronounced at tetracycline treatment. Here, in this case decrease in seedling height may be ascribed to injury caused at cellular level either to gene controlled biochemical or physiological process or acute chromosomal aberration or both, whereas inhibitory effect at lower doses may be either due to mitotic arrest or mitotic delay resulting in growth retardation.

The injury of seedling height is one of the commonest parameter used in evaluating the effectiveness, efficiency and specificity of mutagens in the study of mutagenesis. Studies already made and reported in relation to differential and combined treatment of various groups of mutagens (a) dose dependent reduction (Shaikh *et. al*., 1983) and (b) stimulation in seedling growth at lower doses. Reduction in the seedling height may be due to inhibition of protein synthesis or disruption of mechanisms of gene action controlling its synthesis in the embryonic cell and preventing the entry of cells at G1 to further phases of cell cycles. This may result into either inhibition of emergence of root or shoot or growth retardation.

Chromosomal damage is also supposed to be one of the most common factors for retardation in growth. Root and shoot showed different degree of sensitivity may be due to the facts that the growth of root depends on the cell division whereas that of shoot on cell elongation. More growth retardation in

root and shoot at M1 generation and less effect at M2 is due to the effect of antibiotics which damage more in M1 but persists up to M2 in very dilute states.

The effect of physical and chemical mutagens on plant height has extensively been studied (Shaikh *et. al*., 1983) in different plants and in all the cases on adverse effect of these has been reported. In garlic (Choudhary *et. al*., 1980), in Anethum (Deshmukh, 1981)**,** in *Plantago ovate* (Dube *et. al*., 1981). It has been reported that the magnitude in reduction of height is a function of disc i.e. higher the dose greater is the reduction. Reduction in the plant height with the use of different physical and chemical mutagens is supposed to be the consequence of a number of factors i.e. induced destruction of plant, production of diffusible growth reducing substances, changes in specific activity of enzymes, inhibition of DNA synthesis, chromosomal aberration and reduction in the number of internodes which either may be due to the reduction in cell length without any alteration in cell breadth or reduction in cell number (Gottschalk *et. al*., 1983).

Yield is one of the most common factors for the selection of desired genotypes towards positive and negative direction. Increased, decreased and unchanged variance with mean for number of pod/plant, number of seeds/plant and seed weight/plant as a result of different treatment with mutagens in leguminous crop has been extensively studied (Shaikh *et. al*., 1983; Pathirana *et. al*., 1983). The increased mean at M2 than M1 but still below the control could be assigned to the recovery effect which is a consequence of an elimination of bad genes after selfing. The increase in both mean and variance has suggested unidirectional occurrence of mutations in quantitative traits. The increase in variance reveals the existence of increase in genotypic variability which offers an opportunity of artificial selection following the treatments and also suggests the occurrence of higher frequency of micro-mutations.

Antibiotics which functions like other physical and chemical mutagens induce chromosomal damage, disturb mitotic index and effect the relative frequency of prophase, metaphase, anaphase and telophase. Cell division involves a number of biochemically different processes alongside the chromosome duplication and segregation and thus the chemical which interferes with any of the cytological or biochemical events may induce cytological abnormalities i.e. clumped metaphase, fragments, bridges with or without fragment in anaphase, erosions, telophase bridges and micronuclei.

Decline in prophase is an indication of prevention of entry of cells into division after G_2, antimitotic activity and induction of chromosome aberrations. The increase in metaphase and anaphase and decrease in telophase illustrates the prevention of cell cycle at the end of anaphase and embryonic cell at different stages of interphase at the time of treatment (Amer *et. al*., 1974). Higher frequency of abnormalities at M1 and less at M2 is suggestive of the fact that the chromosomal abnormalities produced at M1 due to direct action of chemicals which go on decreasing in subsequent generation and shows elimination. From the observed different rates of elimination of the various aberrations it can be concluded that the rate of elimination depends on the type of aberration, dose and ploidy. All the antibiotics used were noticed to produce a large number of abnormalities but variability in the degree of damage were different. This may be either due to variation in permeability of the solution or its degree of penetration or dissimilar mode of action.

With the increase of dose there is a corresponding increase in the frequency of aberrations. A single dominant gene is capable of causing meiotic irregularities resulting in partial or complete failure of chromosome pairing (Amer *et. al*., 1974; Grover *et. al*., 1980).

One of the commonest features of the mutagenic treatments is pollen sterility which is associated with the induction of chromosomal changes involving translocation, inversion and deletion. These abnormalities are eliminated during succeeding cell division. Some of the abnormalities remained undetected during mitosis and persist up to gamete formation till M1, M2 or subsequent generation and consequently, cause pollen sterility. Pollen sterility may not only be as a result of the effect of mutagen of polygenic system but also on the genes controlling the meiotic behaviors.

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