Short Communication

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Effect of Epichlorohydrin on the DNA and RNA of Growing Wheat Seedlings

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During the germination of *Triticum aestivum* (wheat) seeds, the effect of epichlorohydrin in the growing wheat shoots at different intervals was found. The study has been carried out on the change of DNA and RNA replicating. This work is being studied spectrophotometerically at PCSIR Laboratories Complex, Karachi.

Living organisms are exposed to various mutagens and carcinogens which are present in the surrounding. The DNA and RNA, components of the nucleic acid are made of nucleotides [1-2]. Chargaff *et al.* [3] developed the methods of synthesis of nucleoside and nucleotides which was first developed by Todd [4]. The study of functions of nucleic acid which has been confirmed [5-6] and has direct correlation to present experiment. Osawa [7] suggested that the RNA of cotyledons of bean is used during growth of seedling and their results indicate that degradation of the storage depends on the presence of the embryo, the nucleic acid changes in the wheat were reinvestigated. Epichlorohydrin, a well known mutagen and carcinogen is widely used as a raw material for production of important industrial chemicals [8], it is reputed to be responsible for production of mutations [9] and their reactions with nucleic acid as biofunctional alkylating agents [10-11], some work, using epichlorohydrin has been done on nucleotide recently [12].

To carry out the determination of effect of epichlorohydrin in growing seeds, the spectrophotometer Photic-100 (Erma Co., Tokyo) petridishes, cotton, wheat seeds (Tandojam Agriculture University of Pakistan), water bath, epichlorohydrin, $HgCl_2$, diphenyl amine, perchloric acid 60% (BDH) and calf thymas DNA (Merck), have been used.

Wheat seeds (*T. aestivum*) family Poaceae (Gramineae) variety Blue Silver, were surface sterilised with 0.1% mercuric chloride solution and washed thoroughly with distilled water to remove mercuric chloride completely.

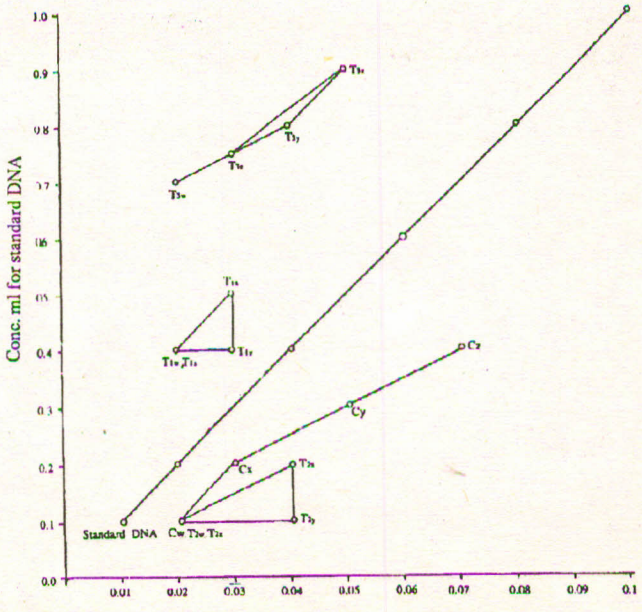
Small quantity of cotton was spread in four sets of petridishes and dishes were labelled as T_1, T_2, T_3 and C (C=control). In T_1 = 0.1%, T_2 = 0.01%, T_3 = 0.001% solution of epichlorohydrin (aqueous) and in C small quantity of distilled water was poured and soaked. The washed seeds were placed in the petridishes in equal number and weight (5 seeds in each dish and average wt. ca. 0.5g).

The dishes were kept covered in dark place for 4 days at room temperature, after interval of 24 hrs, one seed was taken from each petridish and crushed in mortor with pestle in cold methanol. This process was carried out for all seeds individually and the materials was filtered separately.

Insoluble pellets were washed with cold methanol, cold 0.2N perchloric acid and cold ethanol separately, the insoluble pellets were defatted with ethanol: Ether (1:1) at 50° for 30 mins, after that the nucleic acid was extracted with 15 ml of 3% perchloric acid at 70° for 40 mins, absorbance was measured at 260 nm and 290 nm.

A series of standards was prepared by taking 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ml of DNA standard solution and the volume made upto 1 ml with 0.5N perchloric acid, added 3 ml of freshly prepared diphenyl amine reagent [13], heated in boiling water bath for 20 mins. Kept at room temperature, readings were taken at 600 nm against blank (1 ml of 0.5M perchloric acid and 3 ml diphenylamine reagent). Whereas in sample, 1 ml of seed extract + 3 ml of diphenylamine was taken.

The experiment was repeated three times and the results have been calculated (Tables 1-3 and Graph-1). Table1 shows the total nucleic acid present in seeds. The results indicate that control seeds grow normally whereas T_1, T_2, T_3 exhibit a growth decline after 72 hrs. Table 2 shows that DNA in



Graph 1. O.D of Standard DNA and DNA of seed extract.

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control increases normally and T_1, T_2, and T_3 decline after Y position i.e., after 72 hrs. In graph, X-axis is calculated only for concentration of standard DNA and Y-axis for O. D. of standard and seed extract DNA. Standard DNA giving a straight line and control seed extract exhibits small decline at X-position in 48 hrs. but it is more or less nearly a straight line. Whereas T_1 and T_2 seed extracts start from 0.02 and come back to 0.02 O.D. in case of T_3 startings from 0.02 coming towards 0.03 O.D. in T_1, T_1W and T_1Z similar readings have been observed. T_1X and T_1Y show similar results. Where as T_2, T_2W and T_2Z are similar and T_2X and T_2Y are same. The value of CW, T_2W, T_2X, T_1W, T_1Z and T_3W are also the same.

The Tables 2 and 3 show a decrease in the quantity of DNA after 72 hrs except control seed extract but in case of RNA, it is in 72 hrs., T_1 after 48 hrs., T_2 after 72 hrs and T_3 after 72 hrs. The results shows that RNA replicating stops after 72 hrs., except in T_1 where it stops replicating after 48 hrs. As RNA replicating does not take place, therefore, DNA formation stops in control. Therefore, as observed, epichlorohydrin caused mutation which kills the cells afterwards.

TABLE 1.

Time interval (hrs.)	Petridishes	290 nm. O.D.	260nm. O.D.	Difference	Total nucleic acid in µg/dl.
24	C	0.18	0.12	0.06	60
	T_1	0.16	0.11	0.05	50
	T_2	0.16	0.11	0.05	50
	T_3	0.16	0.11	0.05	50
48	C	0.20	0.12	0.08	80
	T_1	0.20	0.15	0.08	80
	T_2	0.21	0.12	0.09	90
	T_3	0.23	0.13	0.1	100
72	C	0.20	0.10	0.12	120
	T_1	0.20	0.12	0.06	60
	T_2	0.21	0.10	0.09	90
	T_3	0.23	0.01	0.12	120
96	C	0.28	0.14	0.14	140
	T_1	0.15	0.11	0.04	40
	T_2	0.17	0.13	0.04	40
	T_3	0.18	0.12	0.05	60

Where C=control, T_1, T_2, T_3=various concentrations of epichlorohydrin of total nucleic acid in µg, given as absorbance difference 100/wt. of seeds.

TABLE 2.

Volume of standard DNA(ml)	DNA absorbance	Absorbance value of seed DNA (600 nm)			
1 ml	1	CW=0.02	T_1W=0.02	T_2W=0.02	T_3W=0.02
0.8 ml	0.8	CX=0.03	T_1X =0.03	T_2X=0.04	T_2X=0.04
0.6 ml	0.8	CY=0.05	T_1Y=0.03	T_2Y=0.04	T_3Y=0.05
0.4 ml	0.4	CZ=0.07	T_1Z =0.03	T_2Z=0.02	T_3Z=0.03
0.2 ml	0.2				
0.1 ml	0.1				

Table shows the O.D of standard DNA & DNA of seed extract, where W=24 hrs., X=48 hrs., Y=72 hrs., & Z=94 hrs. C=control, T_1=0.1%, T_2=0.01%, T_3=0.001% concentration of epichlorohydrin.

TABLE 3.

Control/Extract	Total N.A. in µg	DNA in µg	RNA in µg
CW	60	40	20
CX	80	60	20
CY	120	100	20
CZ	140	140	0
T_1W	50	40	10
T_1X	80	50	30
T_1Y	50	60	10
T_1Z	40	40	0
T_2W	50	40	10
T_2X	90	80	10
T_2Y	90	80	10
T_2Z	40	40	0
T_3W	50	40	10
T_3X	100	80	20
T_3Y	120	100	20
T_3Z	60	60	0

Table shows the quantity of Nucleic acid(N.A.), DNA & RNA which contain ± 0.5% error which may be due to technical or mechanical fault.

Key words: Epichlorohydrin, DNA, RNA and Growing wheat seeds.

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