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A Cytological Analysis of Effects of Water Extract of Gloriosa superba L. on Onion(Allium Cepa L.) Root Tips

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Abstract

Cytological analysis of *Allium cepa* roots treated with *Gloriosa superba* corm extract ,show cytological abnormalities such as polyploidy,binucleate condition,cytoplasmic and nuclear variation,anaphase bridges,anphase clumping etc.

Keywords: *Gloriosa superba,Allium cepa*,polyploidy,vaculation, anaphase bridge, anphase clumping, nuclear cleavage, sticky anaphase, C-metaphase,mutation.

Introduction

There are numerous organic or inorganic chemicals inducing cytological abnormalities in the cells of plants as well as animals. Colchicine one of the famous mitotic poison which induce polyploidy. The corm of *Gloriosa superba* is suggested to contain some amount of Colchicine. Hence the corm extract may induce mutagenesis and polyploidy.

The most important radiomimetic expression of a compound is chromosome breakage. The disruption of hydrogen bond may be the reason for chromosome breakage(Ambrose and Ayengar 1952).Some other scientists pointed out that the action is mainly through an effect on sulphydril groups(Dustin P.,1947).

The alkaloids present in the corm of *Gloriosa superba* L. form the most important radiomimetic chemical (Arun Kumar Sharma and Archana Sharma 1960),most discussed used one being the cochicines. It has been extensively used in inducing polyploidy in many crop plants.

The present study deals with the cytological analysis of the effects caused by corm extract of *Gloriosa superba* L. on onion root tips.

Methodology

The extract of corm of *Gloriosa superba* L. is prepared without adding water .This extract is considered as 100% concentration. From this 50% and 25 % concentrations were prepared by adding 50 and 75 parts of tap water to 50 and 25 part of the extract respectively. Root tips of *Allium cepa* L. is grown up to 1-2cm.long and were treated with 100%,50% and 25% concentrations of the corm extract for 1hour,2 hours,12 hours and 24 hours.

Control is maintained in tap water. The root tip is grown in tap water up to 1-2 cm long.

After treatment the root tips were washed with distilled water. Then root tips were fixed at the time between 9.30am to 10.30 am. Carnoy's fluid used as fixative. The fixed root tips are hydrolysed in 5%

Hydrochloric acid for 3 minutes and squashed in 1% Acetocarmine stain for cytological observations. Mitotic index was then calculated using the formula:-

$$\text{Mitotic Index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells}} \times 100$$

Result and discussion

The main effect produced is on nuclear material. There is change in the behaviour of the chromosomes in mitosis after treatment with corm extract. There is also a significant variation in the mitotic index. Mitotic index is high in cells treated with low concentrations of the extracts, while it shows much reduction in the higher concentrations.

The variation in mitotic index and other changes observed are tabulated in Table1.Nuclear vacuolation is tabulated in Table 2.Nuclear vacuolation increases both with increase in concentration and duration of treatment. The rate of cell division shows significant decrease with increasing concentration and treatment duration. Control shows the maximum mitotic index. Several other changes were noted after treatment such as disruption and disintegration of nuclei, polyploidy cells, binucleate cells etc.

Anaphase clumping ,nuclear coagulation, prophase clumping, cleavage of nuclear material, anaphase bridges, sticky anaphase and C-Metaphase etc were also observed. Sometimes the extract seems to inhibit mitosis.in higher concentrations ,there is complete inhibition(Table 1).Prolonged treatment caused disintegration of root tips. Most common abnormalities observed during the present study were polyploidy cells, binucleate cells and vacuolated cells.

Table 1.Cytological analysis after treatment with different concentrations of *Gloriosa superba* L.corm extract.

Concentration of extract	Treatment period	Mitotic index	Polyploid cells(index)	Binucleate cells(index)
Control		20.61		
25%	1 hr	14.49	2.2	2.6
50%	1 hr	12.17	3.5	2.7
100%	1 hr	8.03	3.5	2.8
25%	2 hr	13.93	3.3	3.0
50%	2 hr	11.41	4.0	2.5
100%	2 hr	6.80	3.8	3.2
25%	12 hr	2.43	3.8	4.6
50%	12 hr	1,22	5.3	5.8
100%	12 hr	……	…..	…..
25%	24 hr	1.91	4.4	6.8
50%	24 hr	0.92	3.0	11.6
100%	24 hr	……	…..	……

Table 2.Nuclear vacuolation after treatment with different concentrations of *Gloriosa superba* L.corm extract.

Concentration of extract	Treatment period	Index of Nuclear Vacuolation
Control		
25%	1 hr	3.02
50%	1 hr	5.98
100%	1 hr	6.32
25%	2 hr	4.52
50%	2 hr	6.63
100%	2 hr	7.67
25%	12 hr	6.94
50%	12 hr	8.13
100%	12 hr	…..
25%	24 hr	8.64
50%	24 hr	14.03
100%	24 hr	…..

A significant amount of cytological damage was induced by the water of *Gloriosa superba* L.Some chemicals may disturb the sulphydril groups of nucleic acids and some others disturb the hydrogen bonds of nucleic acids and still others may affect the oxidation-reduction system within the nucleus (Arun Kumar Sharma &Archana Sharma,1990)mode of action of these chemicals reveal that they possess radiomimetic properties. The alkaloid Colchicine which is a wel known polyplodizing agent cause more or less similar effects within the cell.

The extract mainly affected the nuclear materials. It cause the mitotic arrest in higher concentrations and there is significant reduction of mitotic indexin lower concentrations. Similar effect is produced by Colchicine (Swanson,1957).Polyploid cells found to occur in various treatments They may be formed because chemicals present in the extract may inhibit the formation of spindle and confine the chromosomes within one nucleus, though they their divisions remain unhampered.

The binucleate cells were formed du to the lack of formation of cell-plate and thus cell wall formation. Here the chromosome after duplication moves to opposite poles and form two nuclei, but division of cell in to two daughter cells does not occur.

The division frequency decreases both with increasing concentration and prolonged treatment. This may be because some chemicals present in the extract inhibit the cell division. Cytoplasmic and nuclear vacuolation were the other major abnormalities induced by this extract. Root tips of *Allium cepa* L. treated with *Gloriosa superba* extract show both the types of vacuolations ,mainly nuclear vacuolation. In vacuolated cells nucleus is found on one side and accumulated cytoplasm on the other side .Nuclear material also move to one side of the nuclear membrane leaving a part of nucleus vacant.

Stickiness of chromosomes in metaphase and anaphase were noted and they may be formed by the joining of chromosomal arms. The root tips of *Vicia faba* treated with insecticide, methamidophose show stickiness and clamping of chromosomes. According to Darlington(1962) this may be due to deplomerization of DNA. The elements present in *Gloriosa superba* extract may induce the diplomerization of the complex polymer of DNA molecules. Silk dyeing industry effluent induce mitotic abnormalities in *Allium cepa* root system(Sudhakar,R.,Ninge Gowda K.N.,Venu G.,2001)

The anaphase bridge is another common abnormality found due to this treatment. Anaphase bridge may be formed due to unequal exchange or due to dicentric chromosomes. The dicentric chromosomes may be formed by breakes at the same locus and the fusion of the broken ends of the homologous chromosomes.

Present study revealed the presence of C-metaphase. In C-metaphase, the chromosome become 'chi-shaped".C-metaphase may lead to polyploidy, since the centromeres fail to separate. Cells with C-metaphase were observed in the case of treatment with colchicines (Eigsti and Dustin 1957,Somasekhar et al 1985).

In higher concentration there is complete inhibition of mitosis and the cells were with deeply stained nuclei. There is cell and nuclear disintegration also. This may due to some poisonous substances present in the *Gloriosa superba* L. extract.Curcumin is found to induce mitotic arrest due to spindle abnormality **(**Louise M. Blakemore et.al.,2013)

Thus it can be concluded that the water extract of *Gloriosa superba* L. can induce many cytological abnormalities. Such abnormalities were found to increase with increasing concentrations and also with increasing treatment duration. The extract also has polyplodizing effect. Hence it can be concluded that *Gloriosa superba* L. plant may induce mutation and polyploidy in its neighbouring plants.

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