Indian Journal of Plant Sciences ISSN: 2319–3824 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2014 Vol. 3 (2) April -June, pp. 154-158/Nair
Research Article

EFFECT OF CHLORAMPHENICOL ON PROTEIN SYNTHESIS IN *CHLOROPHYTUM TUBEROSUM* BAKER

***Geetha G Nair**
Department of Botany, RIE, Mysore-6
**Author for Correspondence*

ABSTRACT

An attempt has been made here to find out the effect chloramphenicol has on the protein synthesis of the germinating pollen of *Chlorophytum tuberosum*. Three factors have been studied viz., effect on the percentage of germination, effect on the rate of tube growth and division of the generative nucleus and its behaviour during germination. Though 0.3% of chloramphenicol affects %age of germination and the rate of tube growth, it has no inhibitory effect on the division of the generative nucleus. 0.6% of chloramphenicol certainly inhibits the division of the generative nucleus as no male gametes are formed after 20 hours of treatment. This is an indicator that protein synthesis which takes place before cell division is inhibited.

Keywords: *Chloramphenicol, Chlorophytum tuberosum, Protein Synthesis*

INTRODUCTION

Chloramphenicol is an antibiotic which inhibits protein synthesis by inhibiting translation during protein synthesis. It inhibits the synthesis of proteins in bacteria but the synthesis of deoxyribonucleic acid and ribonucleic acids are not affected (Hahn, 1959). An attempt has been made here to find out the effect chloramphenicol has on the protein sysnthesis of the germinating pollen of *Chlorophytum tuberosum*. Three factors have been studied viz., effect on the percentage of germination, effect on the rate of tube growth and division of the generative nucleus and its behaviour during germination. For all the three processes viz., of tube growth germination and division of the nucleus, enzymes are needed. Enzymes are proteinous in nature. So, when chloramphenicol inhibits translation, protein synthesis and therefore the production of enzymes (eg., cellulase for the cell wall) is inhibited. Chloramphenicol (CHL) whose effects were further characterised, each markedly reduced total RNA synthesis and protein synthesis (Johnson *et al.*, 1976). CHL destroys a wide spectrum of bacteria, including many gram-positive and gram-negative cocci and bacilli. It is now commonly prescribed by veterinarians. The antibiotic chloramphenicol inhibits the uptake of cations and anions by higher plant tissues when supplied at high concentrations (1-2g/l). Since chloramphenicol specifically inhibits the synthesis of protein in bacteria, it has been suggested that this finding indicates a close connection between salt uptake and protein synthesis in plants (Ellis, 1963).

Chlorophytum tuberosum Baker belongs to family Liliaceae and is being used in the indigenous systems of medicine as a galactogogue and aphrodisiac. It is being sold in the market under the common name *safed musali*. The white tuberous roots of this plant are the medicinally useful parts (Patil and Deokule, 2010). Safed musali is found in natural forests right from east Assam to Gujarat. *Chlorophytum tuberosum* is a perennial herb with long cylindrical tuber like root tubers. Leaves are sessile, usually falcate, margins usually crisped; flowers are in simple racemes. Scape is longer than the leaves; perianth -6, free, petalloid, white, macrescent and rarely deciduous, stamens -6, hypogynous, free, anthers as long as the filaments; ovary globose, 3-celled, ovules 4 or more in each cell, style filiform and stigma small; fruit is a capsule. In India about 8 species of Safed musali are reported. Out of them only *Chlorophytum borivillianum, Chlorophytum arundinaceam* and *Chlorophytum tuberosum* are commercially collected by

our tribes from the forest. *Chlorophytum borivillianum* is the only species which is under commercial cultivation.

Some of the important species are as follows-

1. Chlorophytum borivillianum

2. Chlorophytum arundinaceum

3. Chlorophytum tuberosum

4. Chlorophytum malabericum

5. Chlorophytum attenuatum

and

6. Chlorophytum breviscapum

The tubers are used in Ayurvedic medicines; it contains about 27 alkaloids, steroid saponin (2-17%), polysaccharides (40-45%), carbohydrates, proteins (7-10%), minerals, vitamins etc. White musli or Dhauli musli is used for the preparation of health tonic used in general and for curing impotency as they are rich in glycosides (www. jeevanherbs.com). It has spermatogenic properties.

MATERIALS AND METHODS

The plant was selected for study bearing in mind its medicinal properties and the ease with which it can be grown in a green house for experimental purposes. *Chlorophytum tuberosum* was grown and the flowers collected from the green house /botanical gardens.

A few plants were grown in pots and the flowers were plucked every day at 3.40 p.m. The anthers were separated and kept for anthesis in the fridge. The next day the pollen from these anthers were used for preparation of control slides and treatment slides.

The petri-dishes were provided with moist blotting paper to maintain the proper conditions of humidity. Also, a slide was placed inside to prevent the wet blotting papers from touching the experimental slides placed above it.

A 10% culture medium was used for mounting the pollen. 300 mg of calcium nitrate, 200 mg of magnesium sulphate, 100 mg. of potassium nitrate and 100 mg. of boric acid were weighed out and dissolved in 100 ml of distilled water. This gives the stock solution. To 1 ml of the stock solution 10% sucrose solution was added.

Chloramphenicol solutions were prepared by dissolving 3 mg and 6 mg of the substance in 10 ml. of distilled water to get 0.3% and 0.6% concentration of chloramphenicol respectively.

A set of controls were kept and two treatments of 0.3% and 0.6% concentrations were done. In the set of controls, first of all the pollen was mounted on the culture medium on a slide in a petri dish. The slide was observed after every 5 minutes and the time required for germination was found out. After that the percentage of germination of the pollen after every 15 minutes (for 2 hours and 30 minutes) was found out. For finding out the percent of germination, the slide was treated with alcohol acetic acid: 3:1 fixative, then stained directly in acetocarmine after allowing it to dry up. With the help of the same slides the length of the pollen tubes and the behaviour and division of the generative nucleus was observed

For each of the treatments a control was kept. As before, slides with culture medium plus pollen were kept in a petri-dish and after every 5 minutes till 30 minutes the medium was treated with chloramphenicol. The petri-dishes were then kept in the fridge and the effect of chloramphenicol was observed after staining the slides with acetocarmine after 12 hours. Here also the percentage of germination, the length of the pollen tubes and the division and behaviour of the nucleus was observed.

RESULTS

All the observations were tabulated as shown in the tables 1-6.

Table 1: % age of germination in Controls

Time	%age of germination
0 mins	-
5 mins	-
10 mins	-
15 mins	-
20 mins	-
25 mins	-
30 mins	-
45 mins	60.5%
60 mins (1 hour)	50.1 %
75 mins	50.7%
90 mins	50.4%
105 mins	79.4%
120 mins	80.8%
135 mins	60.7%
150 mins	80.3%

Table 2: %age of germination in experimentals
Treatments

Time (mins)	%age germination at 0.3% concentration	%age germination at 0.6% concentration
0 mins	70.3%	80.3%
5 mins	75.7%	80.1%
10 mins	70.07%	50.9%
15 mins	71.6%	70.09%
20 mins	70.8%	60.2%
25 mins	60.9%	40.2%
30 mins	70.8%	70.9%
control	70.6%	70.7%

Table 3: Length of the pollen tubes
Controls

Time (mins)	Tube length
45 mins	67.20 µ
60 mins (1 hr)	124.95 µ
75 mins (1 hr 15 mins)	64.05 µ
90 mins (1 hr 30 mins)	119.70 µ
105 mins (1 hr 45 mins)	153.30 µ
120 mins (2 hrs)	217.35 µ
135 mins (2 hr 15 mins)	175.35 µ
150 mins (2 hrs 30 mins)	186.90 µ

Table 4: Length of pollen tubes
Treatments

Time (mins)	Tube lengths at 0.3% concn.	Tube lengths at 0.6% concn.
Control	291.54 µ	198.66 µ
0 mins	210.01 µ	276.06 µ
5 mins	254.388 µ	238.908 µ
10 mins	238.908 µ	152.22 µ
15 mins	243.52 µ	219.30 µ
20 mins	233.748 µ	190.236 µ
25 mins	199.176 µ	228.588 µ
30 mins	253.356 µ	175. 956 µ

Table 5: Division and behaviour of the generative nucleus
Controls

Time (mins)	Behaviour of generative nucleus (GN)
45 mins	Div of GN in cell itself, Veg nucleus not degenerated.- GN intact and VN degenerating –GN entering the tube and VN degenerating-Both GN and VN (Veg. nucleus) inside the tube- GN dividing and VN degenerating
60 mins	GN dividing in the cell and VN degenerating-GN dividing and entering the tube and VN completely degenerated-GN dividing in the tube and VN degenerating
75 mins	GN dividing and the VN degenerated almost completely
90 mins	GN entering the tube and VN degenerating in the cell-GN dividing in the pollen tube
105 mins	GN dividing in the tube
120 mins	GN just entering the tube-GN dividing inside the tube
135 mins	GN at the tip of the tube in the metaphase stage-GN has divided into male gametes
150 mins	Division of the GN complete with the formation of 2 male gametes

Table 6: Division and behaviour of the generative nucleus
Experimentals

Time (mins)	Behaviour of GN at 0.3% concn.	Behaviour of GN at 0.6% concn.
Control		GN just dividing
0 mins		GN has not started division
5 mins	As the effect of this concentration on the %age of germination and the pollen tube lengths was negligible, the effect on the division of the generative nucleus was not studied	GN has not started division; in some it is just starting division
10 mins		GN intact and in some just dividing
15 mins		GN intact and in some just dividing
20 mins		GN has not started division
25 mins		In some the GN has not divided-in some it has reached the anaphase stage of division
30 mins		In some GN in metaphase stage of division-in some division of GN is over

DISCUSSIONS

Tables 1 and 2

Effect on the Percentage of Germination

a) In the set of controls as the time increases there is a certain gradual increase in the percentage of germination

b) At 0.3% concentration the percentage of germination varies from 60-75% which is lesser than the %age of germination after 2.5 hours (i.e. 80.3%) in the set of controls

c) At 0.6% concentration of chloramphenicol the percentage of germination was lesser than that of the control at 2.5 hours.

Tables 3&4

Effect on the Rate of Tube Growth

a) In the set of controls, with increase in time, an increase in the rate of pollen tube growth is seen but with some variations.

b) At 0.3% concentration treatment of chloramphenicol, the tube lengths are lesser than that of the control of the same concentration (i.e. 291.54 µ), the maximum lengths of the tubes in the treatment being 254.388 µ.

c) At 0.6% concentration of chloramphenicol, the maximum tube length is got at 0 time (276.06 µ) and there is no further increase in length with increase in time.

Tables 5 and 6

Division and Behaviour of the Generative Nucleus

a) In the controls, with increase in time the generative nucleus gradually divides and starts passing into the tube where finally after 2.5 hours at the tip of the tube it divides to give rise to two male gametes.

b) Though 0.3% of chloramphenicol affects %age of germination and the rate of tube growth, it has no inhibitory effect on the division of the generative nucleus.

c) 0.6% of chloramphenicol certainly inhibits the division of the generative nucleus for even after allowing the pollen to germinate for 12 hours after the treatment, there was no formation of male gametes (male gamete formation after 2.5 hours in the set of controls)`

The minimum time taken for germination of the pollen of *Chlorophytum tuberosum* has been found to be 45 minutes. Before that there is no germination at all.

In the set of controls as the time increases there is a gradual increase in the percentage of germination. An increase in the rate of pollen tube growth is seen but with some variations. As for the division and behaviour of the generative nucleus it is found that with increase in time the generative nucleus gradually divided and starts passing into the tube where finally after 2.5 hours, it undergoes division to give 2 male gametes at the tip of the tube.

d) The %age of germination of pollen when treated with 0.3% concentration of chloramphenicol varies from 60-75%.which is lesser than the %age of germination after 2.5 hours in the set of controls. So, it can be said that this concentration of chloramphenicol has some effect on %age pollen germination.

c) The tube lengths in the 0.3 % concentration treatments is lesser than that of the control for the same concentration which is 291.54µ, the maximum value of tube length in the 0.3% concentration treatment being only 254.388 µ. But it is also seen that though this concentration affects the %age of germination and the rate of tube growth, it has no inhibitory effect on the division of the generative nucleus.

d) The %age of germination due to 0.6% concentration treatment of chloramphenicol is lesser than that of the control at 2.5 hours, therefore, there is an effect on the %age of germination for the treatments were allowed to germinate for more than one hour. In case of tube lengths, the maximum tube length is obtained at 0 time and further with increase in time, the tube lengths are not greater than 270.06 µ.

0.6% certainly inhibits the division of the generative nucleus. Even after allowing the pollen to germinate for 12 hours, there is no formation of male gametes, whereas male gamete formation is seen after 2.5 hours in the set of controls.

REFERENCES

Ellis RJ (1963). Chloramphenicol and Uptake of Salt in Plants. *Nature* **200** 596-597.

Hahn FE (1959). *Biochemistry of antibiotics, Proceedings of the Fourth International Congress of Biochemistry* edited by KH Spitzy and R Brunner **5** (Pergamon Press, London) 164.

Johnson Lowell B, Niblett Charles L and Lee Richard F (1976). Effects of inhibitors of RNA and Protein Synthesis on Aspartate Transcarbamylase Activity in Etiolated Plant Tissue. *Plant Physiology* **58**(2) 232–236.

Patil VN and Deokule SS (2010). Pharmacognostic study of *Chorophytum tuberosum* Baker. *International Journal of Ayurveda Research* **1**(4) 237–242.