Effect of Calcium Salts on *Allium cepa* Chromosomes

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Interesting results have been obtained by exposing chromosomes to various chemicals and physical factors (Bose 1967, Darlington 1947, Herich 1969, Kihlman 1966, Manna 1976, Mehra 1974, Moutschen 1978, Pantulu 1967, Raghuvanshi 1978 and Verma 1978). Some workers have tried to evaluate the effect of deficiency of certain elements on chromosomes (Das 1976).

The role of Ca^{++} was clearly indicated in relation to chromosome structure by Cohn (1969) and Ambrose (1973).

The present work was taken up to see the effect of calcium salts namely $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and CaCl_2 on chromosomes. As calcium is one of the binding blocks of the nucleoprotein, it must have some relationship with the chromosomal activities in the cell.

Materials and methods

Ten healthy bulbs of *Allium cepa* were germinated in distilled water. Six bulbs with a good number of healthy roots were selected. Five bulbs were kept over specimen tubes filled with 0.021 M, 0.042 M, 0.063 M, 0.084 M and 0.105 M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ salt solutions. The sixth bulb was allowed to grow in distilled water filled in a specimen tube. This served as a control for the experiment.

Two root tips were cut out every 24 hrs from each bulb. Root tips were washed in distilled water, fixed in 1:3 acetoalcohol, stained and squashed as usual. This process was repeated every 24 hrs for 120 hrs. Chromosomal studies were made from temporary preparations. Photographs were taken to show features of interest.

The recovery of the cells could not be studied as the roots decayed after 120 hrs in all the treated bulbs.

The cells were studied from five different fields of focus under the microscope. The number of cells affected were counted (without putting emphasis on the stages of division of the cells) and the total number of cells under the same focus was also counted. The percentage of cells affected were then deduced.

Observations

It was noted that only after 24 hrs of treatment cells were affected. Higher concentrations (0.084 M and 0.105 M) produced C-mitosis, chromosome breakage, stickiness within 24 hrs of treatment. In some cases degeneration of chromatin started. The lower concentration showed the same effect but in lesser number of

cells. After longer duration of treatment all cells were affected. The higher concentration treatment arrested the growth of roots only after 72 hrs of treatment. There was complete degeneration of chromatin material in the cells after 120 hrs of treatment.

Control showed normal behaviour of chromosomes upto 200 hrs. After that stickiness developed in the chromosomes.

The peripheral cells showed the effect of the chemicals first and after laps of more

Figs. 1-6. Effect of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solutions on *Allium cepa* roots. 1-2: Effect of 0.021 M solution after 24 hrs of treatment. 1, higher number of chromosomes at its breakages at metaphase. 2, higher number of chromosomes and its fragments at anaphase and late anaphase. 3, effect of 0.042 M solution after 24 hrs of treatment. Fragmented chromosomes at metaphase. 4-5: Effect of 0.063 M solution after 24 hrs of treatment. 4, fragmented chromosomes at metaphase. 5, fragmented chromosomes at anaphase. 6: Effect of 0.084 M solution after 24 hrs of treatment. 6, stickiness in chromosomes at metaphase.

time, more and more inner cells were affected. The degeneration of nuclei started after 96 hrs to 120 hrs in case of lower concentrations while in higher concentrations only after 72 hrs of treatment.

Table 1. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ M. solutions showing % of cells affected after every 24 hrs of treatment

Time hrs	0.021 M	0.042 M	0.063 M	0.084 M	0.105 M	Nature of effect
24	10	15	25	35	45	See photographs
48	25	40	55	60	75	
72	45	65	75	80	100	
96	75	90	95	100	100	
120	100	100	100	100	100

Figs. 7-8. 7, delayed coiling of chromosomes. 8-9: effect of 0.105 M solution. 8, degeneration of nuclei after 24 hrs of treatment. 9, all the nuclei showing degeneration after 120 hrs of treatment. 10: Control in distilled water after 288 hrs. 10, normal metaphase plate.

A separate series of experiment was run with the same molar strength of CaCl_2 salt solution. The results were visibly the same. As such no separate table and photographs were necessary.

Discussion

The result obtained clearly indicated that calcium ions played important roles

in bringing about chromosomal breakage (by affecting the phosphate bridge) stickiness, C-mitosis, fragmentation, degeneration of chromatin material and complete elimination of nuclei from the cells (Sharma and Sharma 1972, Sharma 1976).

The structure of chromosome is basically dependant on the Ca^{+} , and thus any change in the concentration of Ca^{+} ions in the solution results in the imbalance of the nucleoprotein complex, leading towards a number of abnormal changes in the chromosomes.

The reduction in the size of the chromosome was partly due to elimination of heterochromatin portions and partly due to strain caused on account of tighter coiling of the nucleoprotein complex in the presence of higher ion concentration of Ca salt. In some of the cells stickiness and uncoiled chromosomes were noted at metaphase as if the higher concentration affected the coiling pattern adversely after the optimum concentration was reached.

Summary

The effect of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and CaCl_2 salt solutions were studied on the chromosome of *Allium cepa* roots. The effects were found to be pronounced in higher concentrations even after 24 hrs. The same results were found in case of lower concentrations but only after longer laps of time period. It was noted that coiling pattern of chromosomes had some relationship with calcium ion concentration in the solution.

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