

NOTE
SEPARATION OF LENTIL ROOT TIP CHROMOSOMES
BY COLCHICINE¹

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ABSTRACT

Various concentrations of colchicine and treatment durations were used on lentil (*Lens esculenta* Moench) root tip cells to separate chromosomes. Squash technique and light microscope were employed. Chromosome separation was affected by different concentrations of colchicine. Maximum number of cells with well-separated chromosomes were obtained with a concentration of 0.05 g/l of colchicine. The best treatment duration was 18 hr.

INTRODUCTION

Chromosomes in cells of many crops including lentil (*Lens esculenta* Moench) are very congregated. In order to spread the chromosomes, different chemicals and techniques have been used. Hermsen *et al.* (2) placed the flowers at 2 C to spread out the chromosomes of *Solanum* cell. Moh (3) obtained enlargement and shortening of chromosomes using *p*-dichloro benzene and heat on bean cells. Sharma and Sharma (5) used karyotype analysis to study the cytological effect of synthetic and natural coumarins of several plants, especially those in monocotyledonous group. Gimenez-Martin and Lopez-Saez (1) employed 8-oxyquinolein as a pretreatment to study the chromosome structure of *Scilla nonscripta* cells.

Sarafi (4) studied the effect of colchicine on bean root tips. He showed that separation of chromosomes was dependent on colchicine concentration and duration of treatment. He found that a solution of 0.01 g/l of colchicine for 6 hr was the best treatment

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to separate the chromosomes in bean. The effect of colchicine on separation of lentil root tip chromosomes is reported in this note.

MATERIALS AND METHODS

An Iranian cultivar (Isfahan No. 11136) of lentil (*Lens esculenta* Moench) from the pulse collection of Agricultural College of Tehran University was used in this study. Seeds were soaked in water for 2 hr and then were placed between filter papers in a petri dish. Ten ml of colchicine with appropriate concentration were added. Colchicine concentrations of 1, 0.1, 0.05, 0.01 and 0.00 g/l were chosen for the experiment. The petri dishes were placed in an incubator at 37 C for 18, 24, 36 and 48 hr. The design of the experiment was a randomized complete block with four replications and each experimental unit consisted of 15 lentil seeds in a petri dish.

Following the treatments, five normally germinated seeds were chosen at random from each dish. The selected seeds were washed with distilled water. About 6 to 8 mm of root tips were cut and then the squash test was applied. The specimens were fixed, hydrated, rinsed, stained with Schiff's reagent, rinsed again, mounted and studied under a light microscope.

To observe the prepared samples under the microscope, a square was drawn on the ocular lens to provide a smaller field. Total number of cells, cells at division stages and cells with well-separated chromosomes within the square were determined. Five square fields were selected from each replication. Due to differences among the data obtained, all calculated percentages were converted to $\text{Arc sin } \sqrt{\text{percentage}}$.

RESULTS AND DISCUSSION

The results obtained are given in Table 1. Analysis of data showed that the effect of duration of colchicine and the interaction of duration and concentration were significant at the 1% level of probability. The duration, concentration and their interaction affected the number of cells at division stages of mitosis. However, the effects of these factors were almost similar for prophase, metaphase, anaphase and telophase stages. Metaphase was found to be the best stage to study the chromosome number.

As it is evident from Table 1, the percentage of cells with separated chromosomes was reduced when colchicine concentrations higher or lower than 0.05 g/l were applied.

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Table 1. Percentage of cells with separated chromosomes as affected by different colchicine concentrations and duration of treatments.

Concentration, g/l	Duration of treatment, hr				Mean
	18	24	36	48	
1.00	20.00	15.15	16.66	18.75	17.64 e *
0.10	22.22	21.21	22.22	17.78	20.86 b
0.05	11.12	28.78	22.24	25.43	21.89 a
0.01	20.00	18.18	21.77	15.62	18.89 d
0.00	26.66	17.66	11.12	23.91	19.84 c
Mean	20.00 a	20.20 a	18.80 b	20.30 a	

*- Means in the marginal row or column followed by the same letter are not significantly different at the 1% probability level (Duncan's test).

This is in agreement with the results of Sarafi (4) who showed that clumping of bean chromosomes occurred when high concentrations of colchicine were used. Treatment duration of 18 hr, although not significantly different from 24 and 48 hr, was considered to be the optimum due to its effective speed.

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