Induced Meiotic Reductions in Root-tips

III. Effect of pyrimidine derivatives, and RNA

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This is a continuation of the previous two contributions (Mehra and Dhiman 1986 a, b) and deals with the effect of pyrimidines on the root-tips of *Pterotheca falconeri*.

Thymine, cytosine, cytidine, uracil, uridine monophosphate, as well as RNA were used in these experiments. The effect of thymine here is considered separately because it was somewhat different from that of the other pyrimidines, namely, cytosine, cytidine, uracil, and uridine monophosphate which are considered together; RNA effect is described at the end. A brief preliminary account of the effect of these substances was given earlier (Mehra and Kumari 1982). Treatment and recovery were carried at $20 \pm 1^\circ\text{C}$, along with corresponding controls. pH of the treatment solutions varied slightly due to solubility in dist. water (Table 1).

Results

Thymine

Thymine is not completely soluble in water. A small quantity of sodium hydroxide solution was, therefore, added to dissolve it. pH and temperature were found to play a very important role in thymine treatment.

Because of difficulty in solubility, clear solutions were obtained only at pH 9.3. Thymine precipitated when the solution was brought to neutral pH. Treatments with this oversaturated solution were toxic, even at low temperatures, because of which the seedlings turned brown and died immediately thereafter. Meaningful results were obtained at lower concentrations (35 mM–50 mM) at pH 9.3.

Mitotic index: The results are presented in the form of graphs in Figs. 1a, b. It will be noticed that 54 mM and 60 mM were lethal causing complete cessation of mitotic divisions. There was gradual decrease in mitotic index in concentrations up to 50 mM but beyond this the decrease was sharp. Besides concentration it also depended upon duration of treatment and the recovery period. In 45 mM and 50 mM two hr treatment there was decrease in mitotic index in 6 hr and 24 hr respectively after which it levelled off (Fig. 1b).

"Meiotic reductions": Though thymine was found to be an efficient chromosome breaking chemical (to be dealt with later), it is pertinent in the present context

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Table 1.

Chemical	Mol. Wt.	pH	Conc.
Thymine	125	9.3	35-60 mM
Uracil	112	8	50-60 mM
2'UMP (Na-salt)	369	7	50-60 mM
Cytosine	111	7	50-60 mM
Cytidine	243	7	50-60 mM
RNA (Na-salt)	—	7.2	5-10%

Fig. 1a, b. Thymine treatment. For explanation see text.

to consider its effect in inducing "meiotic reductions". The maximum percentage of these (21.7%) were scored in 50 mM/1 HT/24 hr. (cf. Table 2). Figs. 4, 5 depict such "meiotic reductions" where 3 homologues are observed at each end without any evidence of the functioning of a spindle. Occasionally "meiotic reductions" occurred also at prophase when the chromosomes were still elongated (Fig. 2). A semblance of pairing of homologous chromosomes was also observed (Fig. 3). In the equational divisions that followed "meiotic reductions" spindles were definitely involved as indicated by the orientation of centromeres. Haploid cells with the characteristic complement of 3 chromosomes were observed not infrequently (Fig. 6).

Reductional groupings: Percentage of the sum total of various types of reductional groupings were calculated. The ratio between "meiotic reductions" and reductional groupings of all types at optimal concentration of thymine was found to be 21.74: 17.2 (cf. Table 2).

Cytosine, cytidine, uracil, and uridine monophosphate

With the exception of uracil all the other pyrimidines are freely soluble in water. Uracil is only partially soluble and hence a small amount of sodium hydroxide solution was added to dissolve it. pH was kept at 7 in all cases, except uracil in which it was 8.

Mitotic index: All the four pyrimidines did not effect significantly the number

Figs. 2-6. Thymine treatment. 2, "meiotic reduction" at prophase (45 mM/2 HT/24 HR). 3, pairing of homologues (40 mM/2 HT/96 HR). 4, "meiotic reduction" (40 mM/2 HT/72 HR). 5, "meiotic reduction" (40 mM/2 HT/96 HR). 6, haploid cell with complete complement of A B C (50 mM/1 HT/24 HR). $\times 2640$.

Figs. 7-10. Cytosine treatment. 7, "meiotic reduction" at prophase (57 mM/2 HT/48 HR). 8, "meiotic reduction", note CC homologues still paired at the equator (57 mM/1 HT/48 HR). 9, "meiotic reduction" (57 mM/1 HT/48 HR). 10, equatorial divisions following "meiotic reduction" (60 mM/1 HT/96 HR). $\times 2640$.

of mitotically dividing cells. Mitotic index was independent of concentration and duration of treatment, irrespective of the pyrimidine used, except in uracil treated series where mitotic index decreased in 6 hr and 12 hr, but seedlings in all the concentrations recovered by 24 hours.

Fig. 11. Haploid cell with complete complement of A B C chromosomes (54 mM/1 HT/96 HR). Cytosine treatment. $\times 2640$.

"Meiotic reductions" and reductional groupings: "Meiotic reductions" were noticed in cytosine, cytidine, uracil and uridine treatments in different percentages, in addition to reductional groupings. These were seen in all the concentrations tried and no relationship with the recovery period was noticed. Their percentage, however, decreased with increase in the treatment period.

Segregation of homologous chromosome into two haploid groups was seen at prophase in cytosine treatment (Fig. 7). Here one accessory chromosome is also seen at each end. In Fig. 8, chromosomes A and B are observed at each end while the CC pair is still lying at the equatorial region. Complete separation of the homologues is noticed in Fig. 9 (for cytosine), 12-13 (for cytidine), 17 (for uracil) and 19-20 (for uridine). In Fig. 20 there are three accessories also of which one lies at the upper end and two at the lower.

Pairing of homologous chromosomes was occasionally noticed. Fig. 16 shows pairing of BB, CC chromosomes and AA pair is still in the process (uracil treatment).

Of the four pyrimidines tested, cytosine induced the highest percentage of "meiotic reductions" i.e. 25% (6 anaphases out of 24) in 57 mM/2 HT/48 HR. Maximum percentage of "meiotic reductions" scored in cytidine was 21.47% (32 "meiotic reductions" in 149 anaphases) in 57 mM/1 HT/48 HR, in uracil 20.69% (12 "meiotic reductions" in 58 anaphases) in 60 mM/1 HT/24 HR, and in uridine treatment 23.53% (8 "meiotic reductions" in 34 anaphases) in 50 mM/2 HT/24 HR (cf. Table 2).

Comparing the percentage of "meiotic reductions" and reductional groupings of all types observed in optimal concentrations it became apparent that they occurred in the ratio of 25: 8.32 in cytosine, 21.47: 13.42 in cytidine, 20.69: 23.79 in uracil, and 23.53: 0 in uridine treatments respectively (cf. Table 2).

After the "meiotic reductions" instead of formation of resting nuclei, equational divisions ensued in which there was separation of chromatids, thus simulating the

Figs. 12-15. Cytidine treatment. 12, "meiotic reduction" (57 mM/1 HT/0HR). 13, "meiotic reduction", (54 mM/2 HT/48 HR). 14, four haploid nuclei organised in a cell after second meiotic division (60 mM/1 HT/48 HR). 15, haploid cell with complete complement of A B C chromosomes (50 mM/2 HT/48 HR). $\times 2640$.

second meiotic division of sporocytes (Figs. 10 in cytosine, 18 in uracil, and 21 in uridine). Such divisions always made their appearance after 24 hr recovery. Combinations other than ABC/ABC/ABC/ABC *were never observed*. Fig. 21 (uridine treatment) is very interesting in showing three chromosomes at each of four poles, with the two poles on the right having 3 accessory chromosomes each.

In Fig. 14 a cell from cytidine treatment is observed with four haploid nuclei of equal size.

Figs. 16-18. Uracil treatment. 16, pairing of homologues (50 mM/2 HT/24 HR). 17, "meiotic reduction", note complete haploid set at each end (54 mM/2 HT/12 HR). 18, equatorial divisions following "meiotic reduction", orientation of centromeres of daughter chromosomes is suggestive of involvement of spindles (57 mM/1 HT/72 HR). $\times 2640$.

Four to five haploid cells were observed in cytosine treatment, the same number in cytidine treatment and one in uracil treatment.

Ribose nucleic acid (RNA)

RNA extracted from yeast was used. Its sodium salt was made to dissolve it in water. Owing to its high molecular weight, mM solutions could not be prepared, instead solutions were made in mg/cc. Treatments were given in 1-10% concentration at pH 7.2.

Mitotic index: Treatment with RNA in lower concentration did not much influence the number of mitotically dividing cells. No correlation could be traced between the concentration and mitotic index, but at higher concentration i.e. 9% and 10% in 1 HT and 72 HR-96 HR, mitotic index was considerably depressed compared to the control.

"Meiotic reductions": Of the different concentration used 5% solution in 1 HT/24 HR was the optimal with maximum "meiotic reductions" (cf. Table 2). The homologues segregated to opposite ends in 3:3 groups. In some cells pairing of homologous chromosomes and the formation of pseudo-chiasmata was also observed (Fig. 22). Here there is end to end pairing of the AA pair and longitudinal pairing of the BB, CC pairs.

Fig. 23 shows the next stage comparable to meiosis II. Here four groups of

Figs. 19-21. Uridine treatment. 19, "meiotic reduction" (54 mM/1 HT/0 HR). 20, "meiotic reduction" with one accessory chromosome at the upper pole and two accessories at the lower pole (54 mM/1 HT/72 HR). 21, equational divisions, on the right hand side 3 accessories can be seen at each pole (54 mM/1 HT/72 HR). $\times 2640$.

Figs. 22-26. RNA treatment. 22, pairing of homologues with pseudo-chiasmata (5%/2 HT/48 HR). 23, second meiotic division, note the regular orientation of centromeres of daughter chromosomes (7%/1 HT/24 HR). 24, haploid cell with complete complement of A B C chromosomes (5%/1 HT/24 HR). 25, haploid cell at prophase. 26, mitosis in haploid cell (5%/1 HT/24 HR). $\times 2640$.

Table 2. Percentage of "meiotic reductions"/reductional groupings on treatment with optimal concentrations of pyrimidines and RNA

	Name of the chemical	Optimal treatment	"Meiotic reductions"	Reductional groupings
1	Thymine	50 mM/1 HT/24 HR	21.74	17.2
2	Cytosine	57 mM/2 HT/48 HR	25.00	8.32
3	Cytidine	57 mM/1 HT/48 HR	21.47	13.42
4	Uracil	60 mM/1 HT/24 HR	20.69	23.79
5	Uridine	50 mM/2 HT/24 HR	23.53	—
	monophosphate			
	RNA	5%/1 HT/24 HR	44.86	—

three chromosomes each can be seen. Apart from ABC/ABC/ABC/ABC segregation, other combinations *were never observed*.

RNA treated root-tips also showed haploid cells (Fig. 24). In 5%/2 HT out of 216 division figures scrutinised, 42 showed this reduced number. These could be formed by the laying down of cell plates after first "meiotic reductions", or might be the result of equational divisions following it. The occurrence of haploid cells in prophase (Fig. 25) and at anaphase (Fig. 26) suggests the possibility that the haploid cells themselves may undergo further divisions.

Reductional groupings: Various types of reductional groupings were noticed in concentrations other than 5%. Detailed scoring of percentages of different types of reductional groupings as well as "meiotic reductions" were carried out to see whether or not the latter were merely chance separations. In the optimal RNA concentration the ratio between "meiotic reductions" and reductional groupings was found to be 44.86:0 (cf. Table 1), but in other combinations they occurred in various degrees.

Abstract

Pyrimidines and their derivatives (thymine, cytosine, cytidine, uracil and uridine monophosphate) and RNA have been found to effect "meiotic reductions" in the root-tips of *Pterotheca falconeri* in significant numbers, followed by second division comparable to what occurs in meiocytes during meiosis. The high percentage of "meiotic reductions" in contrast to reductional groupings of all types taken together in optimal concentrations of these physiological substances is quite meaningful, particularly when taken in conjunction with the results already obtained with purines and their derivatives and DNA (this journal). The significance of these results will be discussed in the next paper of the series.

Literature cited

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