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Chromosome Studies on *Trillium kamtschaticum* PALL. and Its Allies. XXX. Effect of post-temperature-treatment on X-ray-induced chromosome aberrations*

By

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It has been confirmed by many investigators that temperature affects the frequency of chromosome aberrations induced by X-rays. SAX & ENZMANN ('39) demonstrated that low temperature (3°C) during irradiation produces a higher frequency of chromosomal and chromatid-type exchanges than high temperature (30°C) does. In addition, when low temperature was applied after irradiation certain increase in the aberration frequency was shown, although there was an exceptional case of chromatid-type aberrations when irradiated at early prophase. Further confirmatory evidences for the increase of X-ray-induced aberrations by low temperature were presented by CATCHESIDE & LEA ('45), CATCHESIDE, LEA & THODAY ('46), RICK ('40), FABERGÉ ('40) and so on.

It has been suggested that high temperature accelerates the process of rejoining, and thus it favours restitution which recovers original continuity more than new reunion leading to exchanges. Further approach to an understanding of the rejoining mechanism was made by WOLFF & LUIPPOLD ('55) in their fractionation experiments. They showed that low temperature during the interval between two dose fractions makes the broken ends produced by the first dose stay open and fully interact with those by the second dose, thus resulting in more frequent formation of interchanges.

In the previous preliminary study with the PMCs of *Trillium*, however, MATSUURA et al. ('61) obtained a conflicting result indicating that the low

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temperature-treatment of long duration rather brings about a decrease in the yield of two-hit aberrations. The researches along this line are now conducting in our laboratory, and the present paper constitutes a part of them, dealing with the effect of post-low and post-high temperature-treatments of various periods of duration.

Material and Methods

The plants of *Trillium kamtschaticum* PALL. were irradiated late in October (31/Oct.), when the last premeiotic division had completed in their anthers and meiocytes seemed to be at early prophase. X-ray exposure was made at 180 kV, 6 mA. The dose of X-rays was 30 r and the temperature during irradiation was 17°C. Immediately after irradiation one group of the materials was placed in the thermostat set at 27°C, and the second one was kept in a refrigerator at ca. 0–2°C. The rest third group was, as control, planted in pots and transferred into a frame whose condition is nearly the same as natural one. The durations of treatments were 1, 6, 18 and 48 hours in thc case of high temperature, and 6 and 18 hours in the low temperature. After the temperature-treatments the plants were transferred into the frame. Late in January the meiocytes entered the first metaphase.

The PMCs were fixed and stained with aceto-carmine after the 4 : 2 : 1 mixture solution of 0.33 mol of KCl, $NaNO_3$ and CH_3COONa was applied on them for $1\frac{1}{2}$ to 2 minutes, in order to obtain clear figures of chromosome structure. Observations of X-ray-induced chromosome aberrations were made with the first metaphase (MI) and anaphase (AI) cells.

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Results

The main types of chromosome aberrations detected at MI in the present experiments were isochromatid breaks (B″), chromatid-chromatid translocations (T″), chromatid-chromosome translocations (T‴), and minute fragments (mCo). Moreover a few of chromatid breaks (B′) and chromosomal translocations (T⁗) were observed. The chromatid breaks were omitted from scoring because of fear that a large proportion of them might have escaped from detection, particularly before the parallelization of chromatids (cf. MATSUURA '40).

Some particular configurations, as described by MATSUURA & HAGA ('51), were met with, though they are of rather infrequent occurrence, which were interpreted on the basis of "Neo-two-plane" theory as originating from different modes of two-by-two opening-out of chromatids at diplotene after an isochromatid breakage and the sister reunion (Fig. 1). To these isolocus aberrations the symbol iB″ will be given.

The frequency of each type of aberrations mentioned above, is shown in

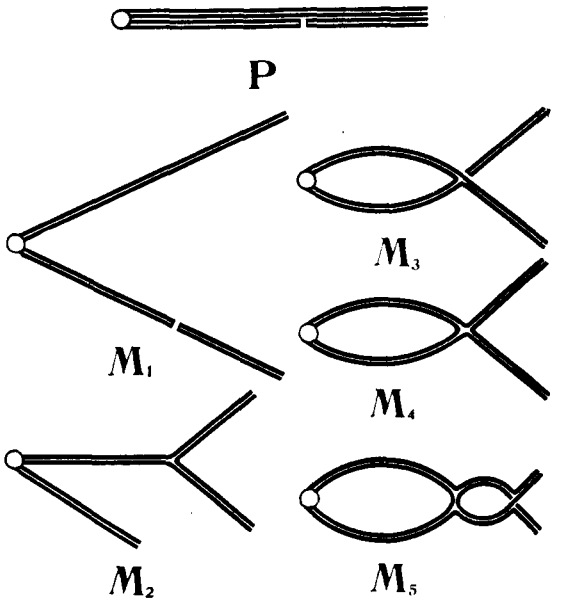

Fig. 1. Diagrammatic representation of various patterns of recombination to be raised from different modes of diplotene opening-out of one affected chromosome pair. Here only one of the two arm pairs of a bivalent is shown, left-handed circles indicating paired kinetochores. P: Pachytene pairing of a normal homologue with an affected chromosome which has been broken into two pieces. M_1-M_5: Five configurations after diplotene opening-out. Hereinafter r and e represent reductional and equational modes of opening-out respectively and the hyphen the position of breakage, the kinetochore being oriented to the left-most. Then each figure is represented as follows: M_1: r-r; M_2: r-e; M_3: e-r; M_4: e-e. M_5 is derived from M_4 when an interstitial chiasma is formed distally to the point of breakage. (ex. MATSUURA & HAGA '51).

Table 1.

The number of breaks involved in exchange—whether chromatid-type or chromosomal-type—is presented as rB and the sum of three types of breaks, B″, iB″ and rB, as *total breaks*. The values in the column of *total breaks* in this table were computed statistically on the basis of the data from two to five plants in each experimental group. The frequency of minute fragments (mCo) is listed separately in the table, because it is not certain whether they are of one-hit or two-hit products. NEWCOMBE ('42) reported that minute fragments include a large proportion of small rings.

In the control material which was transferred to the field condition immediately after irradiation, main types of aberrations were found to be merely

TABLE 1. Effect of post-temperature-treatments on X-ray-induced chromosome aberrations at MI of PMCs in *Trillium kamtschaticum* PALL.

Treatments	No. of cells observed	Frequency per 100 cells					
		B″	iB″	rB	Total breaks	Exchanges	mCo
Control	485	14.0	3.7	19.8	37.7	9.9	2.5
1 hr at 27°C	139	16.5	2.9	7.9	28.2	5.0	0.7
6 hrs at 27°C	256	16.8	2.0	9.0	25.4	4.7	1.6
18 hrs at 27°C	269	13.4	2.2	7.1	23.0	4.1	1.9
48 hrs at 27°C	147	16.3	3.4	8.2	28.7	4.1	2.0
6 hrs at 0-2°C	225	20.9	3.1	24.9	49.2	12.4	2.2
18 hrs at 0-2°C	190	18.4	1.6	9.5	31.5	5.3	3.2

B″=isochromatid break, iB″=particular isolocus aberrations (see text), rB=breaks involved in exchanges, mCo=minute fragments.

B″ and T″, and the former predominated over the latter. In the table, the data of two plants showing extremely deviated frequency, high and low respectively, were omitted, since these values are allowed to be rejectable from the test of homogeneity. The value of *total breaks* was estimated as 37.7 per 100 cells in the control group.

In the group of post-treatment with high temperature, one hour, a remarkable decrease in the frequency of *total breaks* (28.2 per 100 cells) was demonstrated. Similar results were obtained by post-treatments with high temperature, 6, 18 and 48 hours. It is noteworthy that such modification effect of high temperature (27°C) is similar in each case, irrespective of different duration of treatments, and that the period enough for the maximum efficacy is one hour or less. The decrease in the frequency of exchanges is quite remarkable in the series of post-high-temperature-treatments. The frequency of exchanges per 100 cells ranges from 4.1 to 5.0 in the high temperature series, while that in control is 9.9. With regard to the frequency of B″, no distinctive modification effect was recognized.

On the contrary, in the case of post-low-temperature-treatment, the consequence is variable according to different duration of treatment. When treated for 6 hours, the frequency of *total breaks* increases to a remarkable extent. The value obtained from three plants is 49.16, approaching to the value twice as many as those in treatments with high temperature. Exchange yield was 12.4 per 100 cells, and the frequency of simple isochromatid breaks (B″) also increased. These results make a contrast with those of 18 hour treatment, in which both the frequency of exchanges and the value of *total breaks* decreased

to the level lower than those of control, respectively.

Discussion

The present findings that the total frequency of aberrations at MI (excluding B′ and mCo) decreases in the case of post-treatment with high temperature and increases by the treatment with low temperature, 6 hours, are in full accord with previous ones of many investigators. Probably at high temperature restitution from breaks proceeds at a more rapid rate, and a large proportion of broken ends become unavailable for the formation of exchange. The fact that the high temperature (27°C) treatment for only one hour attained to the maximum effect indicates that the process of rejoining is brought to completion within one hour, despite of the especially long duration of the meiotic prophase of the present material under natural conditions.

The present experiment demonstrates striking effect by high temperature treatments modifying only in the frequency of exchanges.

The treatment with low temperature for 6 hours increased the aberrations, especially interchanges. Inhibitory effect of low temperature on the restitution process from breaks may consequently favour the production of interchanges after the inhibition was removed. The increase both in the frequency of B″ and of exchanges clearly indicates that low temperature prevents restitution.

On the contrary, 18 hours' low-temperature-treatment decreased the aberration frequency at MI, resembling the results of a preliminary study on the same material plants which were irradiated late in January when the plants are at the last stage of prophase. The frequency of B″ was higher than that of control and not different from the level of 6 hours' low-temperature-treatment, while the frequency of exchanges was conspicuously lowered nearly to the level of high-temperature-treatments.

In order to obtain a further information about such a discrepancy between these two low-temperature-treatments, a study was made on the frequencies of bridges and fragments at AI and TI (the first telophase) (Table 2).

TABLE 2. Comparison between effects of post-treatments with low-temperature of two different durations on X-ray-induced chromosome aberrations at AI or TI.

Treatments	No. of plants	Total cells observed	Abnormal cells		Fragments		Bridges	
			observed	(per 100 cells)	observed	(per 100 cells)	observed	(per 100 cells)
6 hrs at 0-2°C	2	312	147	(47.1)	166	(53.2)	66	(21.2)
18 hrs at 0-2°C	2	455	188	(41.3)	225	(49.5)	79	(17.4)

The bridge and fragment formation at AI or TI is ascribed to various types of chromosome aberrations observed at MI. The isochromatid breakage followed by sister reunion gives rise to a bridge and a fragment. Bridges may appear either at AI or AII according to the mode of the disjunction of kinetochores (cf. MATSUURA & HAGA '51). A part of exchange may also give rise to bridges and fragments at AI. Comparison of the results from AI and TI cells with those from MI cells, indicates that the frequency of fragments is considerably higher than that of isochromatid breaks at MI, and the frequency of bridges also exceeds that of interchanges observed at MI. This implies that a large proportion of fragments originates from chromatid breaks which cannot be scored accurately at MI, and that certain proportion of AI bridges results from isochromatid breaks followed by sister reunion at the proximal broken ends.

From the fact that the difference in the aberration frequency between the two low temperature groups at AI is not so great as at MI, it will be said with safety that the decrease of interchange in the 18 hours' low-temperature-treatment does not imply that the restitution was promoted. Consequently, it seems likely that this discrepancy is mainly due to the exclusion of simple chromatid breaks in scoring. On the assumption that low temperature of long duration affects the broken ends to lose the ability to form interchange, the decrease of interchange frequency by 18 hours' low-temperature-treatment without decreasing the frequency of simple breaks will be reasonably understood.

Furthermore, it was recently known that the supply of energy is necessary for the process of rejoining of broken chromosome ends (WOLFF & LUIPPOLD '55, BEATTY & BEATTY '59, '60), and also that protein and nucleic acid (especially, DNA) syntheses involve in the rejoining process (WOLFF '59, '60, MATSUURA et al. '62 a, b, c & d, TAYLOR et al. '62). Since the processes of these energy supply and protein or nucleic acid synthesis are enzymatic, they are, of course, dependent on temperature. The present data strongly support this view of rejoining mechanism.

Summary

The effects of post-treatment with high (27°C) and low (0–2°C) temperatures on the X-ray-induced chromosomal aberrations were investigated mainly at the first metaphase in the PMCs of *Trillium kamtschaticum* PALL.

All the post-treatments with 27°C for 1, 6, 18 and 48 hours were found to be very effective in decreasing the exchange yield, mainly chromatid-chromatid translocations, and the maximum efficacy was shown to attain within one hour after irradiation.

In the case of 6 hours' treatments with 0–2°C, the frequency of exchanges and isochromatid breaks increased to a considerable extent. Low-temperature-treatment of longer duration (18 hours) brought about a conspicuous down in the frequency of exchanges. Considering the anaphase and telophase aberration frequencies in these two low-temperature-treatments, it was assumed that the frequencies of simple breaks, both of chromatid and isochromatid-type, are not different between these two groups. It seems likely that low temperature prevents the breaks from restitution favouring the production of interchanges, while low-temperature-treatment of longer duration leads the broken ends to lose the ability for rejoining, thus resulting in the decrease of interchanges.

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