CHROMOSOMAL ABERRATIONS INDUCED BY A PLANT GROWTH RETARDING CHEMICAL (B-995) IN BARLEY (HORDEUM VULGARE)[1]

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(Received, Dec. 5 1966)

The discovery by Muller in 1927 studying the fruit fly *Drosophila*, and by Stadler in 1928 studying barley, that X-rays can induce mutations, represented a milestone in the history of Genetics. It demonstrated clearly that "artificial transmutation of the gene" could be performed by environmental means. In 1943, Oehlkers showed that a chemical substance (aluminum chloride) could be mutagenic and in 1944, Auerbach and Robson first published their results from studies with mustard gas in which they showed this chemical to be a powerful mutagen that could induce a frequency of both gene mutations and chromosome aberrations as great as that from X-rays. These studies led the way for later ones on the radiomimetic effects of certain chemical compounds, that is, the effects produced by chemicals that are similar to those caused by irradiation. Since then, considerable evidence has been accumulated, primarily as a result of more sophisticated techniques, confirming the fact that the genetic material within the cell is subjected to both structural and functional modification by many chemicals, as well as by ionizing radiations.

In previous reports, we have shown that a number of pesticides, namely, herbicides, insecticides and a fungicide, were highly effective in inducing chromosomal aberrations in both mitotic and meiotic cells of barley (Wuu and Grant, 1966a, 1967a) and mitotic cells of *Vicia faba* (Wuu and Grant, 1967b). One interesting case concerned an individual barley plant derived from seed treated with the herbicide Lorox which had 100% of the pollen mother cells with chromosomal abnormalities or cellular irregularities, and was morphologically normal up to the reproductive stage (Wuu and Grant, 1966b). Observations

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are reported here on the induction of chromosomal aberrations by a plant growth retarding chemical, B-995, along with information from X-rayed material used for comparisons.

Materials and Methods

B-995 is a plant growth regulant which was donated for our study by Naugatuck Chemicals, Elmira, Ontario, Canada. The active ingredient is N-dimethyl amino succinamic acid which constitutes 98% of B-995.

Dry barley seeds (*Hordeum vulgare*, agronomic variety Montcalm) were soaked in an aqueous solution of B-995 containing 500, 1000, and 1500 p. p. m. of active ingredient for periods of 6, 12 and 24 hours. After treatment the seeds were washed thoroughly with tap water and incubated at a temperature of 22°C for 36 hours. Root tips were fixed with Carnoy's fluid (3:1 ethanol—acetic acid) and stained by the Feulgen method. Seeds from the 12 hours—1000 p. p. m. treatment were also sown in pots. Carnoy's fixative and the acetocarmine smear technique were employed in the analyses of pollen mother cells. Seeds harvested from C_1 plants were germinated in Petri dishes, or resown in soil, and observations for both mitotic and meiotic chromosome irregularities were carried out in the C_2 generation in the same manner as for the C_1 generation. In another experiment, a volume of 2 ml. of 500 p. p. m. solution of B-995 was sprayed evenly on five seedlings at the age of two weeks, and meiotic cells of the sprayed plants were later examined. Spraying was carried out in a greenhouse at 25°C and the pots were returned to a cold frame 12 hours later.

For comparative purposes, dry barley seeds were irradiated with 5,500 **R** X-rays which is an exposure equivalent to approximately 50% sterility in barley (Ehrenberg *et al.*, 1961). The seeds were germinated and root tips of the seedlings and flower buds of the immature spikelets were studied for mitotic and meiotic chromosomal aberrations, respectively. Seeds harvested from X_1 plants without any further treatment were germinated, and mitotic and meiotic chromosome analyses were carried out in the X_2 generation. In another experiment, seeds irradiated with 2,000 **R** X-rays were subsequently treated with a 500 p. p. m. solution of B-995 for six hours, and an analysis was made on mitotic behavior in the root tips of the germinating seeds.

Results

1. *The first generation:*

The treatments of barley seeds with B-995 produced chromosome abnormalities in root tip cells ranging from 2.84% to 5.64% for the different concentrations and time periods (Table 1). There was some correlation between the

level of concentration of B-995 used in each time interval and the percentage of affected cells. In the case of the 12 hour treatment in which there was a higher average percentage of abnormal cells (5.09%) than in the 24 hour treatment (4.85%), the difference (0.24%) is not considered significant.

The percentage chromosomal abnormalities found in root tip cells from the X-rayed seeds was 4.93% and 9.15% from the 2,000 **R** and 5,500 **R** exposures, respectively (Table 1).

Table 1. *Percentage of barley root tip cells with chromosome abnormalities as a result of seed treatment with B-995, X-rays, and X-rays and B-995*

Treatment	Time period (hours)	Concentration (p. p. m.)	No. of cells examined	Cells with chromosome abnormalities		Mean % for each time period
				Number	Percentage	
First Generation						
B-995	6	500	264	8	3.03*	3.07
		1,000	211	6	2.84	
		1,500	209	7	3.35*	
	12	500	284	12	4.84*	5.09
		1,000	216	11	5.09*	
		1,500	302	16	5.30*	
	24	500	208	9	4.33*	4.85
		1,000	215	10	4.65*	
		1,500	195	11	5.64*	
X-ray (2,000 **R**)	6	500	284	14	4.93*	
X-ray (5,500 **R**)			470	43	9.15*	
X-ray (2,000 **R**) + B-995			225	19	8.44*	
Control			292	2	0.68	
Second Generation						
X-ray (5,500 **R**)	12	1,000	202	5	2.48*	
B-995			200	1	0.50	
Control			234	0	0	

* The percentages starred are significant at the $P > 0.05$ level.

In the combined X-ray (2,000 **R**) and B-995 (500 p. p. m., 6 hours) treatment, 8.44% of the root tip cells were found with chromosome abnormalities, a percentnge approximately equivalent to the sum of the individual X-ray (4.93%) and B-995 (3.03%) treatments. The majority of the chromosomal abnormalities observed in the root tip cells were metaphase fragments and anaphase fragments (Fig. 1) and bridges (Fig. 2).

In the analyses for chromosome irregularities in meiotic cells of the first generation the highest percentage was found from the treatment with the 5,500 **R** exposure of X-rays (3.91%), whereas seeds treated with B-995 (12 hours, 1000 p. p. m.) produced a percentage of chromosomal abnormaties (0.36%) not much greater than that observed in control (0.22%) plants (Table 2). The percentage of microsporocytes with chromosome abnormalties (0.65%) found after the seedlings were sprayed with B-995 (500 p. p. m.) was only slightly greater than that found from plants originating from seed treatment. Chromosome aberrations in pollen mother cells as a result of B-995 treatment are shown in Figs. 3 and 4 and from X-ray treatment in Figs. 5 and 6.

Table 2. *Percentage of pollen mother cells with chromosome abnormalities resulting from seed treatment with B-995, X-rays and seedlings sprayed with B-995*

Treatment	Time period (hours)	Concentration (p. p. m.)	No. of plants examined	No. of cells examined	Abnormal cells	
					Number	Percentage
First Generation						
X-ray (5,500 **R**)	12	1,000	5	460	18	3.91*
B-995 (seed treatment)			5	533	2	0.36
B-995 (seedlings sprayed)		500	3	308	2	0.65
Control			4	454	1	0.22
Second Generation						
X-ray (5,500 **R**)	12	1,000	4	397	5	1.26
B-995 (seed treatment)			5	615	5	0.81
Control			4	408	2	0.49

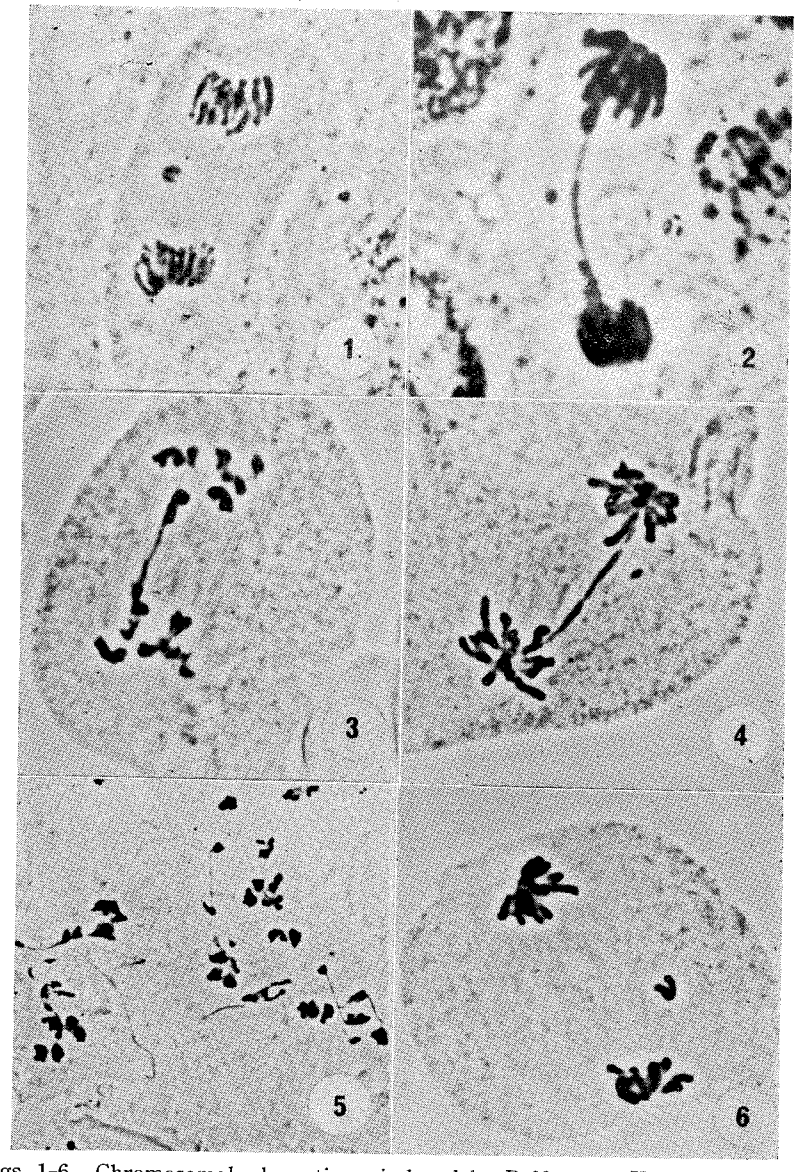
* Significant at the $P<0.05$ level.

2. *The second generation:*

There was an extremely low transmission of chromosomal aberrations in C_2 root tip cells (0.50%) and pollen mother cells (0.81%) in the second generation plants. In the case of the X-irradiated barley, the percentage chromosomal aberrations in X_2 root tip cells (2.48%) and pollen mother cells (1.26%) was considerably higher than those in the second generation from the B-995 treatment, but much lower in comparison with the results from the X_1 generation.

Discussion

The efficiency of B-995 in inducing chromosomal aberrations was considerably greater in root tip cells than in pollen mother cells. This observation is in agreement with our previous studies on the cytological effects of certain



Figs. 1-6. Chromosomal aberrations induced by B-995 and X-rays in root tip and pollen mother cells of first generation barley plants. The treatments are given in brackets.

Fig. 1. Late anaphase root tip cell with a fragment (B-995, 12 hr., 1500 p. p. m., seed soaking) × 4400.

Fig. 2. Late anaphase root tip cell with a bridge and a fragment (B-995, 6 hr., 500 p. p. m., seed soaking) × 5990.

Fig. 3. Pollen mother cell at AI with a single bridge (B-995, 12 hr., 1000 p. p. m., seed soaking) × 1880.

Fig. 4. Pollen mother cell at AI with a bridge and a fragment (B-995, 500 p. p. m., seedling spraying) × 3360.

Fig. 5. Pollen mother cell at MI with chromatin threads and fragments (X-ray, 5,500 **R**, seed irradiation) × 2150.

Fig. 6. Pollen mother cell at AI with a lagging chromosome (X-ray, 5,500 **R**, seed irradiation) × 1480.

pesticides (Wuu and Grant, 1966a, 1967a). The effect of B-995 on meiotic chromosome behavior as revealed by the small percentage of abnormalities scored in the C_1 (0.36%) and C_2 (0.81%), however, was not significant over control.

The occurrence of chromosomal aberrations in meiotic cells varied greatly from plant to plant in both the B-995 and X-ray treated plants. For example, of five X_1 generation plants for which meiotic analyses were made the percentage of chromosome aberrations found was 0, 1.49, 2.78, 4.70 and 15.38%, respectively. Factors which may be responsible for the variation in chromosome abnormalities Among plants have been discussed previously (Wuu and Grant, 1967a).

The low incidence of chromosomal aberrations in pollen mother cells of B-995 treated plants and the low transmission of somatic chromosomal aberrations from the C_1 to the C_2 generation would indicate that the danger of causing a modification of the genetic constitution of B-995 treated plants is considerably less serious than that from seeds treated with X-rays at the dosages employed. Furthermore, the concentrations of B-995 applied in the present study were approximately equal to or slightly higher than that recommended for commercial application (Naugatuck Chemical Company Technical Data Bulletin, February, 1962). From these facts, we might consider that the beneficial use of B-995 in practical plant management would outweigh the possible harmful effects which might be induced, especially since the incidence of meiotic chromosome abnormalities was extremely low. It should be noted, however, that the production of mitotic chromosome abnormalities from seed treatments with B-995 were with one exception statistically significant and that the production of any chromosomal aberrations may produce mutations. Therefore, any chromosomal aberrations are a threat to the genetic constitution of the organism within which they are produced and, subsequently, to their descendants. Chromosomal aberrations are only one type of change which B-995 may induce. The possibility exists that point mutations, which might be traced down to single base substitutions, may also be produced by B-995, but a study of this aspect remains to be carried out.

From a consideration of the percentages of chromosomal abnormalties induced in root tip cells by a combined treatment of barley seeds with B-995 and X-rays (8.44%) and those from B-995 (3.03%) and X-ray (4.93%) treatment alone, it may be concluded that an additive effect was exerted by these two agents when they were applied consecutively. It is generally believed that the localization of aberrations on the chromosomes from X-ray induction is non-specific, whereas, those induced by chemicals have usually a certain degree of specificity (Kihlman, 1966). Such an occurrence might account for part of the additive effect observed in the present study. In addition, the dosages of the X-rays and B-995 used were probably not high enough to cause the maximum

number of aberrations possible, so that the breaking points induced by X-rays and B-995 would not necessarily overlap. Thus one might expect that a combined treatment would produce more chromosomal aberrations than one with either agent separately. Such an explanation, for the additive effect would exclude one in terms of interaction. No information is available on the latter possibility.

Summary

1. Barley seeds treated with B 995, a plant growth retarding chemical, with a concentration of 500, 1,000 and 1,500 p. p. m. for 6, 12 and 24 hours, had chromosomal aberrations in root tip cells of the C_1 generation which ranged from 2.84 to 5.64%. Some correlation existed between the concentration of B-995 applied and the percentage of cells with chromosomal abnormalities observed.

2. Seeds irradiated with 2,000 and 5,500 **R** X-rays for comparative purposes, had 4.93 and 9.15% root tip cells of the X_1 seedlings with chromosomal aberrations.

3. The percentage of pollen mother cells with chromosomal aberrations after seed treatment with B-995 in both the C_1 (0.36%) and C_2 (0.81%) generation plants was very low and the percentages obtained were only slightly greater than those found for controls (0.22%). A similar result was found in an analysis of pollen mother cells after seedling spraying (0.65%).

4. For the 5,500 **R** X-ray treatment 3.91% and 1.26% of the meiotic cells of the X_1 and X_2 generation plants, respectively, were found with chromosomal aberrations.

5. Chromosomal aberrations in root tip cells of the C_2 generation were extremely low (0.5%) indicating a low transmission of chromosomal aberrations.

6. For the 5,500 **R** seed exposure 2.48% of the root tip cells of the X_2 seedlings possessed chromosomal aberrations.

7. An additive effect was observed after a combined treatment of barley seeds with X-rays (2,000 **R**) and B-995 (6 hrs, 500 p. p. m.) in which 8.44% of the root tip cells were found with chromosomal aberrations which is approximately the sum of the individual X-ray (4.93%) and B-995 (3.03%) treatments.

B-995 (一種抑制植物生長之化學物質) 在大麥 (*Hordeum vulgare*) 細胞中所誘致之染色體構造上的變化

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爲了探討若干農用藥物對植物細胞內遺傳物質可能發生之影響，作者等在過去數年內曾對一系列之殺草劑，殺蟲劑及殺菌劑等作廣泛之研究，其結論爲此等藥物均可有效地導致細胞內染色體構造上的變化。在本文內，一種抑制植物生長的化學物質 B-995被用作誘變劑以處理大麥種子，觀察其對根端細胞及花粉母細胞染色體形態、構造及細胞分裂行爲之影響，並以之與X光處理種子所得結果作比較，其要點爲：1. 大麥種子經由三種濃度 (500, 1,000, 1,500 p. p. m.) 和三種時間 (6, 12, 24小時) 的 B-995 水溶液浸漬後，平均有2.84-5.64% 的第一代根端細胞帶有不正常的染色體。異常細胞百分數的大小與 B-995濃度的高低間有某種程度的正相關在。在第二代根端細胞 (僅觀察 1,000 p. p. m.—12小時一個處理) 中，帶有異常染色體的百分數很低 (0.5%)。2. 由B-995 (1,000 p. p. m., 12小時) 處理後所生成之植物，在其花粉母細胞中，無論第一代 (0.36%) 或第二代 (0.81%)，均只有少數細胞帶有不正常的染色體，二者的百分數僅較未處理者 (0.22%) 略高。3. 用 2,000 R 和5,000 R 的X光照射大麥種子，其第一代根端細胞分別有 4.93%和 9.15%帶有異常染色體。在第二代中 5,500 R 也有2.48%的根端細胞帶有此等變異。4. 經 5,500 R X光照射處理，第一代及第二代花粉母細胞帶有染色體異常者分別爲 3.91%與 1.26%。5. 大麥種子先照以 2,000 R X光再浸以 500 p. p. m. B-995 水溶液歷 6 小時，所誘致之異常細胞百分數 (8.44%) 約爲由X光 (4.93%) 和 B-995 (3.03%) 單獨誘致者之和。(摘要)

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