Cytological Effects of Single and Combined Treatments with X-rays, IAA and GA₃ in *Allium cepa*

Suniti Kumar Mandal and Rabindra Krishna Basu

Cytogenetics Laboratory, Bose Institute, Calcutta 700 009, India

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Plant growth hormones have been known to influence mitotic index value (Kato 1955, McManus 1960, Humphries and Wheeler 1960, Davidson and MacLeod 1966, Prasad and Das 1977) and to modify radiation-induced chromosomal damage (Guttman and Brown 1959, Magdon 1964, Araratyan and Azatyan 1974, Araratyan and Vardanyan 1976).

The effects of x-rays singly and in combination with IAA and GA₃ on mitotic index and modification of chromosomal damage in root meristem of *Allium cepa* by pre- and post-irradiation treatments with these growth hormones have been reported.

Material and methods

Root meristem of *Allium cepa*, grown from bulbs, formed the material of the present investigation.

Determination of mitotic index

Mitotic index (MI) was determined in control and treatments with 84 and 126 R x-rays, 25 ppm of indole acetic acid (IAA), 50 ppm of gibberellic acid (GA₃) singly and in combination of x-rays and growth hormones.

Root tips from roots of suitable length of control and treated materials were fixed in a mixture of propionic acid and ethyl alcohol (1:3 v/v) 1, 3, 6, 9, 12 and 24 hr after treatment. Temporary squash preparations were made using propionic orcein as the stain. Cells with mitotic stages from prophase to telophase were scored against total number of cells observed in microscopic fields from coded slides arranged randomly.

Modification of x-ray-induced chromosomal damage

Root meristems were treated with different concentrations of IAA and GA₃ before and after treatments with 84 and 126 R x-rays. The materials kept in distilled water served as control.

Pre-irradiation treatment: Growing roots were placed in aqueous solutions of 25, 50 and 100 ppm IAA and 50, 100 and 200 ppm GA₃, and distilled water (control) in such a way that only the root tips were immersed in them for 24 hr. After the treatments the roots were thoroughly washed in water. The superficial water was wiped off and the roots were irradiated with 84 and 126 R x-rays. The roots were again kept immersed in water for 24 hr before fixation.

Post-irradiation treatment: In case of post-irradiation treatment the concentrations of growth hormones, the durations of treatment and revival and x-ray doses applied remained the same as in pre-irradiation treatment. Here the roots were first irradiated with x-rays and then treated with the growth hormones.

For scoring of chromosomal aberrations root tip squash preparations were made after fixation in a mixture of propionic acid and ethyl alcohol (1:3 v/v) and staining in propionic orcein. Scoring of aberrations were made from anaphase and telophase stages from coded slides arranged randomly.

Results

Mitotic index: Mitotic index (MI) has been defined as the number of cells in mitosis expressed as a percentage of the total number of cells scored.

The MI value remained almost unaffected after treatment with x-rays and GA_3 . Inhibition of mitosis, however, was observed at six and nine hours and nine hours only after treatment with 126 R x-rays and GA_3 respectively and at all fixation times from six to 24 hr with IAA. Post-treatment with IAA, especially after low x-ray dose (84 R) inhibited mitosis sharply, while with GA_3 the inhibition was restricted from three to nine hours after treatment. Compared with 126 R x-rays the reduction in MI value was observed one and nine hours after

Fig. 1. Mitotic index in root meristem of *A. cepa* after treatments with x-rays, IAA, GA_3 singly and in combination of x-rays and growth hormones. (Level of significance. * $P < 0.05$, + $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$).

treatment with IAA and between three and nine hours after treatment with GA_3 (Fig. 1).

Protection against x-ray-induced chromosomal aberration: The x-ray-induced chromosomal aberrations were comprised of dicentric and other (sticky and chromatid) bridges, fragments (terminal and interstitial), micronucleus and laggard (chro-

Table 1. Chromosomal aberrations after pretreatment of roots of *A. cepa* with two concentrations (ppm) of IAA followed by treatment with two doses (R) of x-rays

Treatment	No. of cells examined	Number of aberration per cell					% cells with chromosomal aberrations $\pm$ S.E.	Per cent protection
		Bridge		Fragment	Micronucleus	Laggard		
		Dicentric	Others					
Water+84 R (control)	4330	0.004	0.064	0.079	0.020	0.013	12.06 $\pm$ 0.49	
25 ppm + 84 R	2375	0.002	0.032	0.045	0.009	0.011	***7.70 $\pm$ 0.55	36.15
50 ppm + 84 R	1950	0.004	0.035	0.054	0.008	0.012	***8.82 $\pm$ 0.64	26.87
Water+126 R (control)	2870	0.007	0.106	0.139	0.019	0.015	20.93 $\pm$ 0.76	
25 ppm + 126 R	2190	0.002	0.038	0.068	0.017	0.011	***9.40 $\pm$ 0.62	55.09
50 ppm + 126 R	3300	0.004	0.059	0.076	0.017	0.019	***12.11 $\pm$ 0.57	42.14

***P<0.001

Table 2. Chromosomal aberrations after pretreatment of roots of *A. cepa* with two concentrations (ppm) of GA₃ followed by treatment with two doses (R) of x-rays

Treatment	No. of cells examined	Number of aberration per cell					% cells with chromosomal aberrations $\pm$ S.E.	Per cent protection
		Bridge		Fragment	Micronucleus	Laggard		
		Dicentric	Others					
Water + 84 R (control)	4330	0.004	0.064	0.079	0.020	0.013	12.06 $\pm$ 0.49	
50 ppm + 84 R	3340	0.004	0.034	0.053	0.005	0.011	***7.90 $\pm$ 0.47	34.49
100 ppm + 84 R	3670	0.003	0.032	0.062	0.008	0.006	***7.43 $\pm$ 0.43	38.39
Water + 126 R (control)	2870	0.007	0.106	0.139	0.019	0.015	20.93 $\pm$ 0.76	
50 ppm + 126 R	4510	0.005	0.055	0.079	0.018	0.014	***11.46 $\pm$ 0.47	45.25
100 ppm + 126 R	4400	0.006	0.063	0.069	0.013	0.009	***10.40 $\pm$ 0.46	50.31

*** P<0.001

Table 3. Chromosomal aberrations after treatment of roots of *A. cepa* with two doses (R) of x-rays and posttreatment with two concentrations (ppm) of IAA

Treatment	No. of cells examined	Number of aberration per cell					% cells with chromosomal aberrations $\pm$ S.E.	Per cent protection	
		Bridge			Fragment	Micronucleus			Laggard
		Dicentric	Others						
84 R + Water (control)	3450	0.003	0.041	0.036	0.013	0.009	7.01 $\pm$ 0.43		
84 R + 25 ppm	2030	0.002	0.020	0.040	0.007	0.010	5.76 $\pm$ 0.52	17.83	
84 R + 50 ppm	2600	0.005	0.028	0.027	0.005	0.006	***4.92 $\pm$ 0.42	29.81	
126 R + Water (control)	2600	0.005	0.090	0.097	0.028	0.018	15.45 $\pm$ 0.71		
126 R + 25 ppm	1800	0.004	0.039	0.080	0.013	0.013	***10.49 $\pm$ 0.72	32.10	
126 R + 50 ppm	4485	0.006	0.062	0.062	0.007	0.005	***9.49 $\pm$ 0.44	38.58	

*** P < 0.001

Table 4. Chromosomal aberrations after treatment of roots of *A. cepa* with two doses (R) of x-rays and posttreatment with two concentrations (ppm) of GA₃

Treatment	No. of cells examined	Number of aberration per cell					% cells with chromosomal aberrations $\pm$ S.E.	Per cent protection	
		Bridge			Fragment	Micronucleus			Laggard
		Dicentric	Others						
84 R + Water (control)	3450	0.003	0.041	0.036	0.013	0.009	7.01 $\pm$ 0.43		
84 R + 50 ppm	3250	0.004	0.026	0.034	0.018	0.007	6.00 $\pm$ 0.42	14.41	
84 R + 100 ppm	3010	0.004	0.027	0.035	0.006	0.006	*5.52 $\pm$ 0.42	21.26	
126 R + Water (control)	2600	0.005	0.090	0.097	0.028	0.018	15.45 $\pm$ 0.71		
126 R + 50 ppm	2110	0.010	0.064	0.056	0.016	0.010	***10.14 $\pm$ 0.66	34.37	
126 R + 100 ppm	4710	0.004	0.040	0.039	0.014	0.012	***7.30 $\pm$ 0.38	52.75	

*P < 0.05, ***P < 0.001

mosome and fragment).

The highest concentrations of IAA and GA₃, 100 and 200 ppm respectively, proved to be toxic as the roots became flaccid and further growth was inhibited. These two concentrations were therefore, discarded. The root meristem cells were free of chromosomal aberration after treatment with IAA but showed 3.0 ± 0.76 per cent aberrant cells after treatment with GA₃.

Tables 1 and 2 would indicate that pretreatment with both IAA and GA₃ significantly ($P < 0.001$) reduced x-ray-induced chromosomal aberrations. The reduction in aberration yield with these two hormones remained the same. The magnitude of reduction in aberrant cells induced by the high dose was more with low concentration (25 ppm) of IAA and high concentration (100 ppm) of GA₃.

Posttreatment also with IAA and GA₃ after exposure to x-ray doses reduced chromosomal aberrations significantly, excepting with the low concentration of both IAA and GA₃ (Tables 3 and 4). The differences in the magnitude of reduction between the two hormones were insignificant generally. The maximum reduction was recorded after treatment with the high dose of x-rays followed by high concentration of both IAA and GA₃ (Tables 3 and 4).

The percentage of protection against radiation damage ranged from 18–55 with IAA and 14–53 with GA₃. Pretreatment provided more protection than post-treatment (Tables 1–4).

Discussion

The data presented here show that IAA and GA₃ influence mitotic index and provide protection against x-ray-induced chromosomal aberrations. Mitotic index has been known to be affected after treatment with various chemicals (Ockey 1957). Even amputation of cotyledons decreases mitotic index of roots (Murin 1976). In the present investigation mitosis has been inhibited significantly with IAA and GA₃ and especially when applied after x-irradiation. Mitotic inhibition in root meristem of *Allium fistulosum* has been demonstrated after treatment with GA (Kato 1955), in *A. cepa* with kinetin, IAA, maleic hydrazide (McManus 1960) and with GA (McManus l.c., Robert 1961). In *V. faba*, however, low concentration of GA enhances whereas high concentration inhibits cell division (Prasad and Das 1977).

Besides influencing mitotic index GA₃ has been found to induce chromosomal aberrations which comprised of sticky and chromatid bridges, fragment, micronucleus and laggard, while IAA treated roots had normal mitosis. The occurrence of sticky bridge in GA-treated roots has been reported in *A. cepa* and *A. fistulosum* (Kato 1955). In *V. faba*, however, stickiness is absent and Prasad and Das (1977) are of opinion that GA-induced bridges are probably dicentric chromatid bridges.

In the present investigation both pre- and post-irradiation treatments with IAA and GA₃ have been found to produce considerable protection against x-ray induced chromosomal aberrations implying that the growth hormones have provided radio-resistance and stimulated restitution. The exact mechanism of action of IAA and GA in providing radioprotection has been much debated. Guttman and Brown

(1959) have demonstrated that IAA has no influence on rejoining of x-ray-induced chromosome breakage in *A. cepa*. In *Crepis capillaris*, however, IAA has been shown to stimulate the beginning of DNA synthesis to provide radiation protection (Araratyan and Azatyan 1974). Ribonuclease can stimulate the chromosome repair system and GA has been demonstrated to be involved in controlling the syntheses of alpha-amylase and ribonuclease in barley (Varner 1964) and wheat (Ananthaswamy *et al.* 1971).

Wolff (1959), Wolff and Luippold (1955) and Paleg and Sparrow (1962) have suggested that GA promotes protein synthesis and causes reduction in frequency of chromosomal aberrations. Inhibition of protein synthesis causes inhibition of RNA synthesis and unbalanced DNA synthesis which is known to offer greater radio-resistance (Maaløe and Kjeldgaard 1966).

Summary

Treatments with IAA and GA₃ singly and especially after x-irradiation produced significant reduction in mitotic index in root meristem of *Allium cepa*.

Pre- and post-irradiation treatments with IAA and GA₃ also showed significant reduction in x-ray-induced chromosomal aberrations. The percentage of protection ranged from 18–55 with IAA and 14–53 with GA₃. These growth hormones were considered to have reduced aberrant cells by providing protection against initial radiation damage and also by stimulating restitution presumably by affecting DNA synthesis and also by stimulating protein synthesis respectively.

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