EFFECT OF INDOLE-3-ACETIC ACID AND KINETIN ON GERMINATION SEEDLING GROWTH AND SOME BIOCHEMICAL CONSTITUENTS IN GAMMA IRRADIATED SEEDS OF CHICK-PEA

HAJRA AZHAR ALI AND SHAGUFTA ANSARI

Department of Botany, University of Karachi, Karachi-75270, Pakistan.

Abstract

Effect of indole-3-acetic acid and kinetin were studied on the germination, seedling growth and some biochemical constituents of chick pea seeds irradiated with 0.2 and 0.7 kgy gamma rays. Exposure to gamma rays significantly inhibited germination and seedling growth. Combined application of IAA and Kinetin however increased germination, root and shoot growth as compared hormones used singly. Root and shoots raised from seed stock after combined treatment of hormones showed pronounced increase in dry matter, reducing sugar and carbohydrate contents as compared to seedlings raised from seeds treated with IAA and Kinetin alone.

Introduction

Gamma rays and other ionizing radiations are gradually increasing in our environment. These are considered as environmental stress and are known to induce varied physiological and biochemical changes in seeds which ultimately manifest in plant growth (Sparrow, 1961; Goud, 1967; Bajaja, 1970; Hussain & Khan, 1971). Exposure of plants to gamma irradiation brings about changes in the endogenous level of growth hormones. Degani (1976) reported increased abscisic acid level in root and leaves of gamma irradiated wheat seedling and suggested that these elevated level of ABA may cause disbalance in the endogenous levels of other hormones. Staikov (1986) found increased levels of IAA and Gibberellin in soybean plants exposed to x-rays while Muira (1974) observed decreased level of IAA in gamma irradiated Avena coleoptile. Very few studies have been carried out to study the interaction between hormones and ionizing radiation. El-Aishy (1976) reported that partial reversal of radiation induced inhibition seedling growth of rice after treatment with GA₂ and IAA. The present report describes the effect of IAA and Kinetin on seed germination, seedling growth and some biochemical constituents of seedlings raised from seed irradiated with 0.2 and 0.7 KGY gamma rays.

Materials and Methods

Chick pea seeds cv. CM 72 with 13% moisture content were exposed to 0.2 and 0.7 KGY gamma rays from CO₆₀ source at Nuclear Institute for Agriculture and Biology, Faisalabad. Irradiated and non irradiated seeds were surface sterilized with 0.2% HgCl₂ and soaked over night at 25°C±1°C in appropriate volume of 10 ppm IAA and Kinetin

Table. 1. Effect of IAA and Kinetin on shoot and root length and on drymass of seedlings from gamma irradiated chickpea seeds.

					0		, -					
Ļ	č		/		% change						% change	Dry
Days	Sho	Shoot length (cm)	(cm)		over non			Root length (cm)	cm)		over non-	
	9	6	12	15	irrad. cont	ıt. in mg.	9	6	12	15	irrad. cont.	in mg.
Control	1.46	7.54	11.14	14.52	1	182±2	2.28	9.60	11.08	17.22		75±1.8
(non-irrad.)	± 0.29	± 0.72	±0.85	±0.73			±0.29	±0.61	±0.61	±1.32		
0.2KGY	1.24	6.92	7.14	9.4	-35.7	100±3.2	1.94	8.72	10.2	11.28	-34.4	41±2.7
	± 0.28	±0.56	±1.31	± 1.63	(45)	(45)	±0.14	±0.49	±0.15	± 0.82		(-45.3)
0.2KGY+IAA	1.82	7.32	8.2	10.88	-25	136±4.1	2.4	9.46	10.76	13.46	-21	71±3.1
	±0.46	±0.74	±0.94	± 2.63		(-10)	±0.26	±0.35	±0.48	+1.8		(-5.3)
0.2KGY+	1.68	7.56	9.03	10.36	-28.6	130±1.8	2.48	9.36	11.36	11.42	-33.6	40.2±4
Kinetin	±0.25	±0.53	±1.25	±0.59		(-25)	±0.34	+0.5	±0.65	±2.5		(-46.5)
0.2KGY+IAA+	2.26	8.80	12.46	16.62	±14.4	*183±3.4	2.76	9.74	16.96	18.82	±9.2	*115±5.1
Kinetin	±0.29	€8.0∓	98.0∓	±1.68		(+0.5)	±0.35	±0.84	∓0.8	±2.2		(+53.3)
0.7KGY	1.02	5.5	5.94	6.14	-57.7	46+2.7	99	7.94	0.8	8.62	49.9	18+2.0
	±0.16	±0.41	±0.74	€8.0∓	· ·	(-74.7)	±0.21	±0.4	±0.32	±1.07		(-75)
0.7KGY+IAA	1.74	8.9	7.18	7.68	-47.1	45.4±5	1.84	8.22	9.3	10.82	-37	21 ± 2
	±0.25	±0.57	±0.8	±1.17		(-73.6)	±0.36	±0.46	±0.49	±0.87		(-72)
0.7KGY+	1.6	7.18	8.7	9.12	-37.1	46.6+2.8	2.34	8.19	8.29	9.94	-52.4	18.2±2.8
Kinetin	±0.34	±0.62	±0.57	±1.32		(-73.6)	±2.2	±0.45	±0.19	+1.1		(9/-)
	6	i	,	1			;	,	,		,	•
0./KGY+IAA+ 2.22 Kinetin ±0.24	2.22 ±0.24	7.64 +0.52	11.08	13.02	-10.3	*132±4.2	2.62 +0.25	9.08 +0.5	14.28 +0.69	16.24	-5.6	70±4.2 (±6.6)
i.						(· · · · · · · · · · · · · · · · · · ·			\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	20.1		

Fig. in parenthesis denotes percentage (+) increase or (—) decrease. 'Asterisk' show significant increase in drymass of shoot and root.

(Sigma) solutions separately and in combination. For comparison the irradiated and non irradiated seeds were soaked in distilled water and 25 seeds from each treatment were placed in 10 cm dia. Petridish lined with double layer of Whatman No. 1 filter paper. Seeds were allowed to germinate in the dark at 22°C±2°C. Emergence of radicle was taken as the criterion of germination. There were 4 replicates of each treatment.

Seedling growth: Five germinated seeds from each treatment together with control were grown in small pots fitted with nylon gauze containing half strength Hoagland nutrient solution. Plants were grown at 25±2°C day and 20±2°C night temperature with 14 hrs dark and 10 hrs light period of 6000 lux. Root, shoot length and their dry matter was recorded at different stages of growth.

Extraction and estimation of reducing sugar: One gram of root and shoot tissues from each samples were ground in 10 ml distilled water and centrifuged at 6000 rpm for 10 min. The supernatant was used for the estimation of reducing sugar as described by Nelson & Somogyi (1944). Total carbohydrate was estimated by anthron method of Yemm & Willis (1956).

Results

High dosage of gamma rays 0.7KGY reduced seed germination by 27% as compared to 15% reduction in 0.2KGY treatment (Fig. 1). Application of IAA and Kinetin alone did not show any significant effect on inhibition caused by gamma rays but the combined application of these hormones significantly (P<.001) reversed the inhibition. Treatment of seeds with a combination of hormones significantly reversed the gamma rays induced inhibition in shoot, root length and dry matter content of seedling (Table 1). This effect was more pronounced in 0.2KGY where the shoot, root length and dry matter showed an increase over non irradiated control. The amount of reducing sugar and total carbohydrate however decreased significantly in root and shoot (Fig. 2 & 3) both at 0.2 and 0.7 KGY gamma rays. Combined application of (1AA + Kinetin) hormones increased the amount of reducing sugar and total carbohydrate (Fig. 2 & 3) substantially as compared to separate use of hormones.

Discussion

The results demonstrate that irradiation of chick pea with gamma rays inhibits germination (Fig. 1). This inhibition is considerably reversed by the combined application of IAA and Kinetin. Gamma rays possibly influence some early metabolic processes (Kurobane, 1979; Levitt,1980) and related enzymes which regulate the process of germination. The increase in percent germination of chick pea recorded after the combined application of IAA and Kinetin may possibly be due to the activation of these processes by the action of these hormones (Gordon, 1957, Machev, 1975).

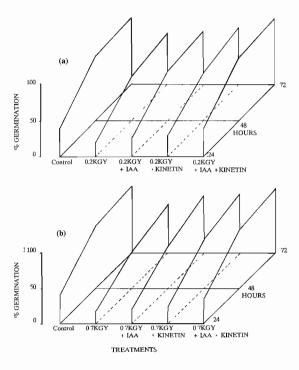


Fig. 1. Effect of growth hormones on germination of chick pea seeds exposed to (a) 0.2 KGY (b) 0.7KGY gamma rays. Data is an average of four replicates.

Gamma rays also inhibited (shoot, root length and dry mass of seedlings and reduced the levels of reducing sugar and carbohydrate both at 0.2 KGY and 0.7 KGY treatments. Exposure to gamma radiation possibly brings about changes in the growth regulators and alteration in glucose metabolism which ultimately contribute to the inhibition of seedling growth and dry matter. (Gordon, 1957; Jordon & Haber, 1974; Degani, 1978; Inuo, 1980).

Use of IAA and Kinetin in combination considerably reversed the inhibition of seed-ling growth and enhanced the levels of reducing sugar and carbohydrates in the seedling raised from gamma irradiated seeds. Proper balance of growth regulators and their interrelationship presumably forms the basis of regulatory control of growth and development in plants (Fuchs, 1968; Abrol, 1969). It would appear that combined application of IAA and Kinetin provides a regulatory control of growth and development of seedling by interacting with inhibitors produced by gamma irradiation. (Smith, 1968; El-Aishy, 1976). Alternatively, accumulation of reducing sugars by the combined application of IAA and Kinetin and subsequent translocation of assimilate may play a role in the recovery of seedling growth from radiation damage (Bhardwaj, 1986). It could therefore be concluded that growth hormones play an effective and complex role in radiorepair mechanism (Levitt, 1980) which needs proper elucidation.

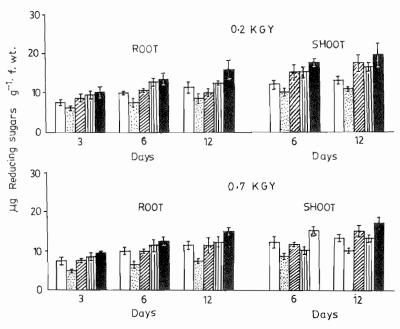


Fig. 2. Effect of growth hormones on reducing sugar in the roots and shoots of chickpea seedlings raised from the seeds exposed to different doses of gamma rays.

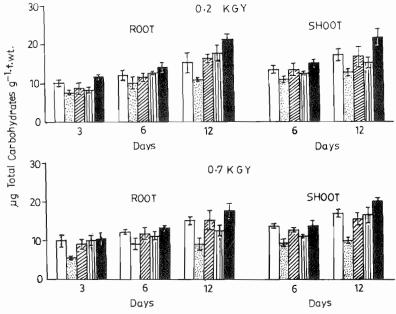


Fig. 3. Effect of growth hormones on endogenous level of total carbohydrate in the roots and shoots of gamma irradiated chickpea seeds.

Control (non-irradiated)		Control (irradiated)	IAA	IIII Kinetin	IAA+Kinetin
--------------------------	--	----------------------	-----	--------------	-------------

Acknowledgements

The authors extend their thank to Director, NIAB for giving permission to irradiate the seeds from CO₆₀ source. Thanks are also extended to Professor Dr. Jamil Ahmed for the help in the preparation of the manuscript and to Dr. Shahid Shaukat for the statistical evaluation of the data.

References

- Abrol, Y.P., G.S. Sırohi and S.K. Sınha. 1969. Reversal of inhibitory effects of gamma rays on seedling growth of wheat by the application of IAA, Tryptophane and Zinc. *Ind. J. Exp. Biol.*, 7, 144-116.
- Bajaja, Y.P.S. 1970. Effect of gamma irradiation on growth RNA, protein and nitrogen content. Annals of Bot., 34: 138.
- Bhardwaj, S.N. and V. Verma. 1986. Hormonal regulation of assimilate translocation during grain growth in wheat. *Ind. J. Exp. Biol.*, 23: 719-721.
- Degani, N. and C. Itai. 1978. The effect of gamma radiation on growth and abscisic acid in wheat seedlings. Environ. Exp. Bot., 18: 113-115.
- El-Aishy, S.M., A. Abdalla and M.S. El-Keredy. 1976. Effect of growth substances on rice seedlings grown from seeds irradiated with gamma rays. Environ. Expt. Bot., 16: 69-75.
- Fuchs, Y. and L. Morris. 1968. Effect of Kinetin, IAA and Gibberellin on ethylene production and their interaction on growth of seedlings. *Plant Physiol.*, 43: 2029-2036.
- Gordon, a.A. 1957. The effect of ionizing radiation on plants: Biochemical and Physiological aspects. Quart. Rev. Biol., 32: 3-14.
- Hussain, H. and M.I. Khan. 1971. Effect of gamma radiation on growth, protein and nucleic acid metabolism of wheat seeds. *Pak. J. Biochem.* 4: 61-66.
- Inoue, M., H. Hiroshi and H. Shiro. 1980. Glucose metabolism in gamma-irradiated rice seeds. *Environ. Exp. Bot.*, 20: 27-30.
- Kurobane, I. and H. Yamaguchi. 1979. Effect of gamma radiation on the production and secretion of enzymes, and on enzymes activities in barley seeds. Environ Exp Bot., 19: 75-84.
- Levitt, J. 1980. Radiation stresses in responses of plants to environmental stresses. Academic Press, pp. 283-343.
- Machev, N.P., M. Giacommelli and D. Kekov. 1975. Study of the effect of cytokinins on some biochemical processes in gamma irradiated plants. Fiziol Rast., 1: 12-20.
- Muira, K., T. Hashimoto, and H. Yamaguchi. 1974. Effect of gamma radiation and cell elongation and auxin level in avena coleoptile. *Radiat Bot.*. 14: 207-215.

- Nelson, N. 1944. Photometric adaptation of Somogyi method of determination of glucose. J. Biol. Chem., 153, 375-380.
- Smith, O.E., W. Yen and J.M. Lyons. 1968. The effect of Kinetin in overcoming high temperature dormancy of lettuce seed. *Proc. Amer. Soc. Hort. Sci.*, 93: 444-453.
- Sparrow, A.H., R.L. Gauny, J.P. Mikshe and L.A. Schair. 1961. Some factors affecting the responses of plants to acute and chronic radiation exposures. *Rad. Bot.*, 1: 10-34.
- Staikov, G., I. Kalin and M. Antonov. 1986. Study of the growth Physiological, biological and productive changes in Soybeans due to gamma radiation. *Physiol Rast.*, 12: 65-74.
- Yemm, E.W. and A. J. Willis. 1954. The estimation of carbohydrates in plants extract by anthron. Biochem. J. 57:508.

(Received for publication 13 May 1989)