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EFFECTS OF GAMMA IRRADIATION ON THE AMINO ACID LEVELS IN NORMAL AND OPAQUE-2 MAIZE (*ZEA MAYS* L.) SEEDLINGS.[1]

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Abstract

Seeds of a hybrid corn with normal genotype and of opaque-2 genotype were irradiated at 0 – 200 Gy (10 mGy= 1 rad) of ^{60}Co gamma irradiation. The free and bound amino acid levels of these two genotypes were compared from shoots of 10 days old seedlings.

In control seedlings totals of free and bound amino acid levels were significantly higher in opaque-2 genotype in comparison with the normal genotype. Following exposure, the free amino acid levels of the normal genotype did not show any significant radiation dependent increase or decrease, while in opaque-2 genotype the total levels of free amino acids showed a significant increase at all exposures. In bound amino acids, on the other hand, significant increase in total levels was observed in normal genotype at all exposures, while in opaque-2 genotype decreases were observed at all exposures, except for 100 Gy. Based on these observations opaque-2 genotype appears to be more radiosensitive than the normal maize.

The results are discussed in relation to the genetic differences in the mechanism of protein biosynthesis of the two genotypes

Introduction

Radiosensitivity of a plant is influenced by biological, environmental and radiological factors (Gunckel & Sparrow, 1961). Among different species nuclear and chromosomal variables, morphological organisation and physiological and biochemical state of the organism have been successfully correlated with radiosensitivity (Sparrow, Schwemmer & Bottino, 1971). However, relatively little work has been done on the response of different varieties of a species to radiation, partly perhaps due to the erroneous assumption that they comprise one sensitivity group (Soriano, 1971). Comparison among varieties of barley (Mikaelsen, & Brunner, 1968), tomato (Bianchi, Marchesi & Soressi, 1963), pea (Monti & Donnini, 1968), soybean (Ukai & Yamashita, 1968), rice (Soriano, 1971) and wheat and sorghum (Iqbal, 1980) showed variations in response to irradiation among different genotypes indicating the influence of genetic factors on radiosensitivity. Since varieties in a species may differ in relatively few hereditary traits, differences in their radiation response may be associated with a factor(s) governing such trait(s).

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The present work was therefore conducted to compare the radiosensitivity of two genotypes of maize (normal and opaque-2). The parameter to assess radiosensitivity was the effects of radiation on the concentrations of free and bound amino acids, as it is in the amino acid composition where basic differences in these two genotypes are found

Materials and Methods

For plants with "normal" nitrogen metabolism, a hybrid named Ceje (A 320 x B 320) X (CE 218 x CE pt) from the Plant Breeding Station was chosen. Maize rich in essential amino acids represented the line S_3 of opaque-2 genotype derived from opaque-2 synthetic population, homozygous for the recessive mutant opaque-2 gene.

Irradiation and growth conditions. Seeds of normal genotype and of opaque-2 genotype were equiliberated to a seed moisture content of 10.8%. Seeds of both genotypes were exposed to ^{60}Co gamma rays in a Gamma-Cell 220 (Canada Limited). The seeds were irradiated at room temperature with doses of 0, 50, 100 and 200 Gy (1 Gy=100 rads). The dose rate was 10 Gy/31. 8 sec. Two seperate, independent and complete irradiations were conducted.

For amino acid analysis, control and irradiated seeds were allowed to imbibe distilled water for 6 hours, and then germinated on cotton wool saturated with distilled water for three days in dark in a climatic room at $22^{\circ}C \pm 2^{\circ}C$. Germinated seeds were grown for seven days under uninterrupted fluorescent light (1000 Lx).

Determination of free and bound amino acid levels. The amino acids were determined from shoots of 10 day old seedlings from both genotypes on an automatic amino acid analyser type 6020 (A product of Development Workshop of Czechoslovak Academy of Sciences). The extraction of free and bound amino acids from plants of the two genotypes and other experimental details were carried in a manner reported in an earlier study (Iqbal, Kutacek & Jiracek, 1974).

Results

The free and bound amino acids levels of shoots in ten day old seedlings of normal genotype (+/+) and opaque-2 genotype (O_2/O_2) are given in Tables 1 and 2.

(i) *Free amino acids.* The total levels of free amino acids in opaque-2 at 0 Gy are significantly higher when compared with the normal genotype. The increase in total level is primarily contributed by increases in levels of lysine, histidine, threonine, glutamic acid, glycine, alanine and tyrosine (Table 1). Further, in opaque-2 shoots a gradual increase in free amino acid levels is observed with increasing radiation dose. The maximum increase being at the highest exposure of 200 Gy (Table 1). In normal maize no significant increase either in any individual amino acid or in total levels was observed at any of the exposures, rather a significant reduction in total amino acids level was observed at 100 Gy (Table 1).

Table 1. Effects of gamma irradiation on free amino acid levels in 10-day old shoots of normal (+/+) and opaque-2 ($0_2/0_2$) genotypes of *Zea mays*. (mg/1 g dry wt).

Abbreviation: tr = traces.

	Normal (+/+)					Opague-2 ($0_2/0_2$)				
Dose (Gy)	0	50	100	150	200	0	50	100	150	200
Lysine	0.42	0.29^{-}	0.22^{-}	0.35	0.41	2.18	2.28	5.66^{+}	6.61^{+}	16.74^{+}
Histidine	0.45	tr	tr	tr	tr	3.98	4.05	8.22^{+}	9.30^{+}	23.12^{+}
Aspartic acid	tr	tr	tr	tr	0.79	tr	tr	4.05	tr	10.77
Threonine	tr	0.18	0.11	tr	0.14	2.54	tr	5.59^{+}	4.87^{+}	18.34^{+}
Glutamic acid	0.34	0.60^{+}	0.40	0 43	0.54	6.51	18.11^{+}	12.29^{+}	9.06^{+}	26.84^{+}
Glycine	0.50	0.48	0.33	0.45	0.50	5.08	6.38	4.54	8.69^{+}	17.42^{+}
Alanine	1.32	1.19	0.47^{-}	1.38	1.33	12.30	18.53^{+}	33.48^{+}	30.24^{+}	89.19^{+}
Cystine	0.77	0.41	0.45	0.62	0.63	tr	tr	tr	tr	tr
Valine	0.28	0.27	0.15	0.14	0.22	1.31	1.87	1.51	tr	9.61^{+}
Meythionine	0.21	0.48	0.12	0.29	0.29	tr	tr	tr	5.60	tr
Isoleucine	tr	tr	0.25	0.12	0.20	tr	tr	tr	2.52	tr
Leucine	tr	tr	tr	0.07	0.13	tr	tr	tr	tr	tr
Tyrosine	tr	0.24	tr	0.15	0.19	3.43	5.05	tr	11.93^{+}	14.41^{+}
Amino-butryric acid.	0.32	0.28	0.12	0.25	0.33	1.61	1.92	2.80	4.91^{+}	12.35^{+}
	4.62	4.42	2.62^{-}	4.25	5.70	38.94	58.19^{+}	78.14^{+}	93.73^{+}	238.79^{+}

(a) The aminoacids arginine, serine, proline and phenyl-alanine were found in traces in control and at all doses of irradiation in both genotypes.

(b) Singnificant increases (+) and decreases (–) over control at P=0.05 (Duncan's multiple range test of composite means).

Table 2. Effects of gamma irradiation on bound amino acid levels in 10-days old shoots of normal (+/+) and opaque-2 ($0_2/0_2$) genotypes of *Zea mays* (mg/1 g dry wt).

	Normal (+/+)					Opague-2 ($^{0}2/^{0}2$)				
Dose (Gy)	0	50	100	150	200	0	50	100	150	200
Lysine	12.92	20.54^{+}	22.97^{+}	19.24^{+}	18.00^{+}	12.20	10.98	13.69	13.82	11.53
Tryptophan	0.81	2.99^{+}	1.43	1.41	0.72	35.20	44.95^{+}	58.94^{+}	28.94^{-}	36.49
Histidine	0.40	1.65^{+}	0.84	0.74	0.39	3.00	2.65	4.85^{+}	2.46	3.75
Arginine	11.63	19.05^{+}	20.73^{+}	19.32^{+}	15.75^{+}	8.81	9.56	11.05	10.30	9.98
Aspartic acid	15.20	16.98	23.39^{+}	18.61	21.01^{+}	24.64	25.17	37.07^{+}	26.00	21.18
Threonine	4.50	7.84	8.69	7.31	6.97	7.41	6.23	14.73	8.02	8.38
Serine	1.90	3.28^{+}	3.63^{+}	3.23^{+}	3.05	2.92	2.34	3.84	2.01	1.64
Glutamic acid	17.25	18.79	23.22^{+}	20.38	21.78^{+}	16.70	14.37	23.22^{+}	15.27	13.47
Proline	8.34	13.15^{+}	14.72^{+}	8.02	12.09^{+}	7.30	8.39	9.53	9.27	8.29
Glycine	8.40	13.44^{+}	15.08^{+}	13.09^{+}	12.47^{+}	10.86	8.51	14.89^{+}	10.31	9.98
Alanine	9.87	15.49^{+}	17.54^{+}	15.00^{+}	14.55^{+}	11.33	8.70	16.86^{+}	10.31	11.87
Cystine	2.73	7.37^{+}	8.35^{+}	7.61^{-}	4.45	10.73	7.86	14.02^{+}	6.64^{-}	10.64
Valine	1.03	1.78	2.36^{+}	1.82	1.67	0.22	0.43	0.80^{+}	1.76^{+}	2.15^{+}
Methionine	1.02	1.00	3.02^{+}	2.82^{+}	1.59	1.22	0.55^{-}	2.29^{+}	1.87	1.39
Iso-leucine	10.15	16.05^{+}	18.60^{+}	15.03^{+}	14.85^{+}	11.33	10.61	15.61^{+}	9.47	8.25
Leucine	20.19	24.93^{+}	28.18^{+}	23.57	23.61	17.34	20.11	27.78^{+}	17.49	19.33
Tyrosine	5.00	6.04	7.08^{+}	6.07	5.60	4.03	2.53^{-}	4.48	3.26	2.99
Phenyl-alanine	9.11	14.84^{+}	16.48^{+}	13.59^{+}	13.11	10.39	8.27^{-}	11.77	9.94	8.34^{-}
Total	140.45	205.21^{+}	233.31^{+}	196.86^{+}	191.66^{+}	195.63	192.21	285.42^{+}	187.02	189.65

Significant increases (+) and decreses (−) over control at P=0.05 (Duncan's multiple range test of composite means).

(ii) *Bound Amino acids.* Comparison of bound amino acids in normal and opaque-2 genotypes, shows that at this early phase of ontogenesis there are changes in the levels of several amino acids in opaque-2. For example, amino acid tryptophan, histidine, aspartic acid, threonine, alanine and cystine are significantly higher in opaque-2. However, there is a substantial decrease in levels of arginine, glutamic acid, methionine and leucine in opaque-2 in comparison with normal maize (Table 2).

Contrary to the observations on free amino acids, the post-irradiation levels (total) of bound amino acids in shoots of normal genotype were higher at all exposures (details reported in a previous report: Iqbal, Kutacek & Jiracek, 1974). In opaque-2, on the other hand, increase in total level of amino acids was observed at 100 Gy only, while at 150 and 200 Gy a decrease occured (Table 2). Increase in total level at 100 Gy was significant and was mainly contributed by increases in levels of aspartic acid, threonine, glutamic acid, proline, alanine, valine, iso-leucine, leucine and tyrosine (Table 2).

Discussion

Maize of the opaque-2 genotype contains substantially more essential amino acids than the normal maize. The differences in the composition of the amino acids appear in the corn endosperm (Mertz, Bates & Nelson, 1964). Very little is known about the composition of amino acids in germinating and young seedlings, though differences in the amino acids composition at early ontogenic phase are reported (Sevcenko & Agafonov, 1971). The present investigation, conducted on 10 day old seedlings of opaque-2 and normal genotype Ceje, confirms the observation of Sevcenko & Agafanov (1971), as significantly higher levels of free and bound amino acids were found in opaque-2 genotype in comparison with the normal genotype (Tables 1 and 2). These differences in the amino acid levels are attributed to differences in the nitrogen metabolism (Joliver *et al,* 1970) and more active transamination processes in the seedlings of the two genotypes (Langer, Stransky & Kutacek, 1975)

Following gamma irradiation the response of the two genotypes varied considerably. In normal genotype at all exposures an increase in bound amino acid levels was observed, an indication of an induced protein synthesis (Iqbal, Kutacek & Jiracek, 1974), while in opaque-2 a decrease at all exposures was observed (except for an increase at 100 Gy). From this it appears that the mechanism of protein biosynthesis in opaque-2 genotype is more radiosensitive than the normal maize. This conclusion is substantiated by the observation that in shoots of opaque-2 the levels of free amino acids showed a linear increase with increasing exposures. This could result when the free amino acids are not being incorporated in the porteins, thereby showing a decrease in the concentrations of bound amino acids.

References

Bianchi, A., G. Marchesi, and G.P Soressi. 1963. Some results in radiogenetical experiments with tomato varieties. *Radiation Botany*, 3 333-334.

Gunckel, J.E. and A.H. Sparrow. 1961. Ionizing radiations: biochemical, physiological and morphological aspects of their effects on plants. *Encycl. Plant Physiol., 16:* 555-611.

Iqbal, J. 1980. Effects of acute gamma irradiation, developmental stages and cultiver differences on growth and yield of wheat and sorghum plants. *Environmental & Experimental Botany, 20:* 219-232.

Iqbal, J., M. Kutacek, and V. Jiracek. 1974. Effects of acute gamma irradiation on the concentrations of amino acids and protein nitrogen in *Zea mays. Radiation Botany, 14:* 165-172.

Jolivet, E., M. Nicol, J. Baudet, and Y. Mosey. 1970. Differences dans l incorporation du $^{14}CO_2$ a la lumiere chez de jeunes maize normal et opaque-2. In: *Improving plant proteins by nuclear techniques.* IAEA, Vienna.

Langer, I., P. Stransky, and M. Kutacek. 1975. Differences of cytoplasmic transaminase activity in normal and opaque-2 maize (*Zea mays* L.) seedlings. *Theoretical & Applied Genetics, 46:* 19-23.

Mertz, E.T., L.S. Bates, and O.E. Nelson. 1964. Mutant gene that changes protein composition and increases lysine content of maize endosperm. *Science, 145:* 279-280.

Mikaelsen, K. and H. Brunner. 1968. Effect of fast neutrons and gamma irradiation on seedling and root growth of barley varieties, pp. 79-82. In: *Neutron irradiation of seeds.* II. IAEA, Vienna.

Monti, L.M. and B. Donini. 1968. Respones to chronic gamma irradiation of 24 pea genotypes. *Reduction Botany, 8:* 473-487.

Sevcenko, V.E. and N.S. Agafonov. 1972. Izuceniji fizilogobiochimiceskogo dejstvja gena opejk-2 u izogennych gibridov kukuruzy. *Vesi. Selskochozj. Nauki, 1.* 55-67.

Soriano, J.D. 1971. The response of several rice varieties to fast neutrons. *Radiation Botany, 11:* 341-346.

Sparrow, A. H., S.S. Schwemmer, and P.J. Bottino. 1971. The effects of external gamma irradiation from radioactive fallout on plant with special reference to crop production. *Radiation Botany, 11:* 85-118.

Ukai, Y. and A. Yamashita. 1968. Varietal differences in radiosensitivity with respect to chromosomal aberrations in soybean. *Proc. 12th Intern. Cong. Genet.* Tokyo, *1:* 109.