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EFFECTS OF GAMMA IRRADIATION AND PERIODIC SAMPLING ON PRIMARY-LEAF AMINO-ACID COMPOSITION OF *PHASEOLUS VULGARIS* L. CV. BLUE LAKE*

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Abstract

Phaseolus vulgaris L. cv. Blue Lake seeds exposed to acute gamma radiation dosages of 0.5, 1.0, 1.5, 3.0, 4.5, 6.0, and 7.5 kR. from a 60Co 4500 Ci source were planted in the greenhouse. Primary leaf samples collected at two stages (10 and 15 days after 50% germination in the control group) were used to estimate biochemical stimulation based on amino acid compositions. Radiation and the developmental stage significantly influenced the amino acid compositions. Biochemical processes mediated by enzymatic activities and irradiation are thought to relate to amino acid variations.

Introduction

Plant growth stimulation from irradiating seed has been credited with increased crop yields. Attempts to correlate such increases with plants nutritional quality as human food are limited but, in general, an inverse quantity/quality relationship exists. Such information may become necessary before irradiated plants are used in human food, so biochemical and physiological induced responses should be studied. To date such studies have dealt with respiration (Woodstock & Justice, 1967), nucleic acid biosynthesis (Cherry et al. 1962), free and bound amino acids, and protein nitrogen (Iqbal et al. 1974). Irradiation effects on amino acid compositions however, seldom have been reported (Bourke et al. 1967; Hagen & Gunckel 1958), and formed the basis of the study reported here. *Phaseolus vulgaris* L. cv. Blue Lake was used to assess biochemical stimulation based on periodic foliar analyses.

Materials and Methods

One-year-old *Phaseolus vulgaris* L, cv. Blue Lake seeds at 12.0 percent moisture content were given single gamma irradiation exposures of 0.5, 1.0, 1.5, 3.0, 4.5, 6.0 and 7.5 kiloroentgens (kR) from a 60Co 4500 Ci source, then planted immediately in flats containing a 2:1:1 mixture of soil: sand: peat. The flats were kept in a greenhouse maintained at 29.4 C (day) and 18.3 C (night) in a randomized, complete block design. Twenty-five seeds were planted for each of the 7 treatments, 2 durations (10 and 15 days after 50% germination in control) and 3 replications.

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After each growth period 20 primary leaves were randomly collected from each treatment and replication, dried and ground on a micro-sample mill to pass through a 0.05-cm screen. The ground samples were mixed and stored at 4°C in airtight containers. Standard procedures for moisture and Kjeldahl protein (AOAC, 1970), sample hydrolysis (Waggle et al. 1966), and amino acid analyses (Spackman et al. 1958) were used. Duplicate determinations for each sample from Rep I and II were made. The data were analyzed after correcting amino acid values to 100 percent recovery of Kjeldahl protein, and to a moisture-free basis.

Results

Gamma radiation induced variations in the amino acid content both for treatments and durations (Tables 1-4). Increases were significant ($p \geqslant 0.01$) for histidine, threonine, serine, and isoleucine at some exposures (Table 1). Lysine,

TABLE 1. Mean amino acid compositions; showing increases; in primary leaves of *Phaseolus vulgaris* L. cv. Blue Lake after planting gamma-irradiated seed.

Amino acid*	Content in Control	Range in γ-treated samples	LSD 0.01
Histidine	2.55	2.38 to 2.94	0.16
Threonine	4.98	4.98 to 5.20	0.16
Serine	4.85	4.75 to 5.08	0.19
Isoleucine	4.80	4.85 to 5.00	0.12
Valine	6.23	5.83 to 6.48	0.20

*Amino acid values after correcting to 100 percent recovery of Kjeldahl protein and to a moisture free basis.

tyrosine, phenylalanine, and proline decreased (Table 2), with valine changes being erratic. Cystine and methionine contents were excluded as they were not based on oxidation to cysteic acid and methionine sulfone. Only aspartic and glutamic

TABLE 2. Mean amino acid compositions; showing decreases; in primary leaves of *Phaseolus vulgaris* L. cv. Blue Lake after planting gamma-irradiated seed.

Amino Acid*	Content in Control	Range in γ-treated samples	LSD 0.01
Lysine	6.65	5.60 to 6.75	0.19
Proline	5.20	4.98 to 5.38	0.18
Tyrosine	4.08	3,73 to 4.08	0.14
Phenylalanine	5.88	3.67 to 5.98	0.19

* Amino acid values after correcting to 100 percent recovery of Kjeldahl protein and to a moisture free basis.

acid increases were consistent, as was the arginine decrease from 0.5 kR (Table 3). These consistent variations were significant ($p > 0.01$). Further, our 15-day post-treatment analyses showed that most amino acids decreased between this duration, but lysine, aspartic acid, proline, and phenyl-alanine increased (Table 4). Threonine, serine, glutamic acid, alanine, and leucine changes were not significant.

TABLE 3. Mean amino acid compositions, with consistent variations, in primary leaves of *Phaseolus vulgaris* L. cv. Blue Lake after planting gamma-irradiated seed.

Amino Acid*	Dosages in kiloroentgen								LSD
	0	0.5	1.0	1.5	3.0	4.5	6.0	7.5	0.01
Aspartic acid	10.55	11.95	11.40	11.20	11.55	11.03	12.83	11.53	0.14
Glutamic acid	12.13	12.50	12.40	12.45	12.70	12.60	13.03	12.53	0.16
Arginine	7.88	5.83	5.95	6.28	5.88	6.23	5.33	6.18	0.15

*Amino acid values after correcting to 100 percent recovery of Kjeldahl protein and to a moisture free basis.

Discussion

The aspartic acid increase appears to be partially a function of carbohydrate metabolism, as less carbohydrate reportedly produces less amide (Patel et al. 1970). Aspartic acid may also have originated from glutamate by transamination, as suggested by Sane & Zalik's (1968) observations during barley germination, and asparagine may synthesize from aspartic acid by asparagine synthetase. The glutamic acid increase likely results from transamination reactions. When respiratory activity starts, the transaminase enzyme is capable of forming glutamic acid, alanine, or aspartic acid. Interconversions are an important variable that may occur in the soluble metabolic pools (Thompson & Zalik, 1974). Patel et al. (1970) after reporting variations in amounts of amino acids in groundnut, suggested that interconversions and biosynthesis may be affected by previous seed irradiation. They found that 10 kR. stimulated synthesis of most amino acids; with photosynthesis, carbohydrate metabolism, trans—and de-amination reactions mediated by enzymatic activity influencing the contents. Other undefined processes also may function. The duration induced decreases over the 15 days period (Table 4) seems a result of seedling growth that leads to physiological aging of the primary leaves and initiates translocatory activities.

Our findings indicate that amino acids were altered by seed irradiation and the consistent significant increases may be ascribed to stimulatory effects. On the same experimental material, stimulation effects were additionally measured through morphological and physiological parameters (Mujeeb & Greig, 1976). The morphological parameters generally facilitate evaluation, and are reliable indices despite heterogeneity in the treatment lots (Conger & Stevenson 1969). Such a stimulation was obtained, though the physiological measure (total chlorophyll content) was negatively related with enhancing dosage. The physiological parameter together with the present amino acid criteria may be grouped into the cellular evaluation index. These are better buffered against extraneous variability and hence seem more precise

TABLE 4. Influence of durations (10 and 15 days) on the amino acid)compositions in primary leaves of *Phaseolus vulgaris L.* cv. Blue Lake after planting gamma-irradiated seed.

Amino acid*	Days post-treatment		LSD
	10	15	0.01
Lysine	6.45	6.60	0.01
Histidine	2.80	2.50	0.08
Arginine	6.55	5.83	0.08
Aspartic acid	11.30	11.73	0.07
Threonine	5.08	5.12	NS
Serine	4.94	4.88	NS
Glutamic acid	12.54	12.54	NS
Proline	4.90	5.50	0.09
Glycine	5.60	5.44	0.08
Alanine	6.32	6.37	NS
Valine	6.41	6.13	0.10
Isoleucine	4.96	4.83	0.06
Leucine	9.38	9.71	NS
Tyrosine	4.70	3.22	0.07
Phenylalanine	.550	5.61	0.70

*Amino acid values after correcting to 100 percent recovery of Kjeldahl protein and to a moisture free basis.

indices of evaluating stimulation responses; accordingly greater time is expended for these or related cellular techniques.

The ultimate importance of stimulation resides in attaining significance in qualitative and quantitative traits at plant maturity to an extent that some prediction of directed stimulatory reproducibility can be disseminated. Generally we observe a dilution of induced effects over plant growth, more prone to which are the morphological entities. Significant stimulation measured through cellular parameter/s however, may be more than transient since these parameters are adapt to partition the environmental and induced cellular influence. This leaves an intricately operating metabolic system with lasting ramifications. Whether our observed changes in the various amino acid compositions maintain stimulatory manifestations upto plant maturity can only be speculated upon. After the induced changes, the ratio of essential amino acids to total amino acids had not changed significantly, so chances of inducing adverse nutritional quality in M_1 yields are remote, but definitely worth exploring further.

References

Association of Official Analytical Chemists. 1970. Methods of Analysis, 11th Edn., Washington, DC. 1015 pp.

Bourke, J.G., B.R. Stillings and L.M. Massey. 1967. Free amino acids in gamma irradiated carrots. Radiation Research, **30**: 569-575.

Cherry, J.H., R.H. Hageman and J.B. Hansen. 1962. Effects of X-irradiation on nucleic acids in *Zea mays* (ii) On the level of ribonuclease activity in growing seedlings. Radiation Research, **17**: 740-751.

Conger, A.D. and H.Q. Stevenson. 1959. A correlation of seedling height and chromosomal damage in irradiated barley seeds. Radiation Botany, **9**: 1-14.

Hagen, G.L. and J.E. Gunckel. 1958. Free amino acid levels following gamma irradiation of *Nicotiana glauca, N. longsdorfii* and their inter-specific hybrid. Plant Physiol., **33**: 439-443.

Iqbal, J.,M M. Kutaček and V. Jiraček. 1974. Effects of acute gamma irradiation on the concentration of amino acids and protein-nitogen in *Zea mays.* Radiation Botany, **14**: 165-172.

Mujeeb, K.A. and J.K. Greig. 1976. Growth stimulation in *Phaseolus vulgaris*-L induced by gamma irradiation of seeds. Biologia Plantarum, **18**: 301-303.

Patel, G.M., K.C. Patel and R.D. Patel. 1970. Free amino acids during germination of γ-irradiated groundnut. J. Agr. Food Chem., **18**: 1168-1171.

Sane, P.V. and S. Zalik. 1968. Amino acid, sugar, and organic acid content during germination of Gateway barley and its chlorophyll mutant. Can. J. Biochem., **46**: 1479-1486.

Spackman, D.H., W.H. Stein and S. Moore. 1958. Automatic recording apparatus for use with chromatography of amino acids. Anal. Chem., **30**: 1190-1206.

Thompson, L.W. and S. Zalik. 1974. Free amino acids in rye seedling embryos during vernalization. Can. J. Plant Sci., **54**: 35-42.

Waggle, D.H., D.B. Parrish and C.W. Deyoe. 1966. Nutritive values of protein in high and low protein content sorghum grain as measured by rat performance and amino acid assays. J. Nutr., **88**: 370-374.

Woodstock, I.W. and O.L. Justice. 1967. Radiation induced change in respiration of corn, wheat, sorghum, and radish seeds during initial stages of germination in relation to subsequent seedling growth. Radiation Botany, **7**: 129-136.