Free Amino Acids During Germination of γ-Irradiated Groundnut

The method of circular paper chromatography allowed estimation of 19 amino acids during germination of the control and the γ-irradiated groundnut. The seeds were exposed to 10, 30, 50, 70, 90, and 120 kR dosage levels. Some amino acids which are absent in the ungerminated seeds are detected during the course of germination periods of 1, 3, 6, 12, 20, and 30 days. Biosynthesis of the free amino acid is affected by γ-irradiation. Carbohydrate and photosynthesis have specific effects on the production of some of the amino acids.

The reserve proteins of seeds, considered to be the chief source of energy, are hydrolyzed by the action of various enzymes to amino acids and simpler compounds. Much work has been reported on the study of free amino acids of various seeds during germination. Nandi (1958) reported the effect of darkness and ultraviolet irradiation on the free amino acids of some leguminous seeds. Ultraviolet radiation caused a decrease in almost all the free amino acids during first 24 hr of germination. Action of very large doses of X-radiation on free amino acids, protein, and nucleic acid contents of wheat has been studied by Vasil'ev *et al.* (1960). They noted that irradiation of wheat plants with 300 kiloroentgen (kR) of radiation did not destroy the existing amino acids. Ibragimov *et al.* (1961) reported that the exposure of cottonseeds to large radiation doses (50 million-r) of γ-rays resulted in a partial destruction of the amino acids present in protein and a complete decomposition of all free amino acids except aspartic acid.

The ionizing radiations have varied effects on the reserve protein and free amino acids of oil bearing seeds. The present study describes the variation of the free amino acids during germination of the γ-irradiated groundnut.

EXPERIMENTAL

Germination. A special variety of groundnut AK-12/24 MP, obtained from Junagarh Research Farm (Gujarat State), was exposed to γ-rays from ^{60}Co-source (1000 kc) located at the Bhabha Atomic Research Center (BARC), Trombay, India. The integrated dosage levels used were: 10, 30, 50, 70, 90, and 120 kiloroentgen (kR). The control and the irradiated seeds were germinated for 0, 1, 3, 6, 12, 20, and 30 days in sterilized sand at 25 ± 1° C. Only distilled water, no other nutrients, was added daily in a measured quantity to the germinating seedlings.

Extraction of Amino Acids. At the end of each germination period two sets of 10 seedlings each were removed from the sand, cleaned with distilled water, and, after drying, were defatted with petroleum ether (40° to 60° C) in a Soxhlet apparatus. The defatted plant materials (cakes) were used for the extraction of free amino acids.

To 0.100 g of cake in a centrifuge tube, 15 ml of 75% ethanol were added and homogenized for 10 min in a homogenizer. The mixture was then centrifuged. The supernatant extract was collected in an evaporating dish. The residual cake was extracted twice with alcohol. The extracts were combined with the first one, then evaporated to dryness on a water bath. The residue left after evaporation was dissolved in 1.0 ml of 10% 2-propanol and refrigerated until assayed. The extract of ungerminated control seed cakes was prepared by the same method.

Separation and Estimation of Amino Acids. The procedure adopted for the separation and quantitative determination of the free amino acids is essentially the same as described by Krishnamurthy and Swaminathan (1955).

Solutions of each test sample were applied in small spots, using a micropipette, along the circumference of a circle of radius 2.5 cm drawn at the center of a square Whatman No. 1 filter paper (46 × 46 cm). The required concentration was built up on the paper by repeated spotting of 5 μl quantities after drying. After fixing up a wick at the center, the filter paper was placed in a chromatographic box with the brushlike end of the wick dipping in the solvent system. The chromatogram was irrigated three times in the butanol:water:acetic acid (40:50:10, v/v/v) solvent system. It was irrigated twice in the phenol:water:2-propanol (70:25:5 v/v/v) system. After desired irrigation, the filter paper was dried in a current of dry air and sprayed with a 0.4% solution of ninhydrin reagent in acetone. The chromatogram was then heated at 80° C for 10 min to develop color of the ninhydrin reacting substances.

Each stained band of the unknown and the corresponding known amino acid was cut out of the chromatogram. These were extracted in separate test tubes containing 5 ml of 75% ethanol saturated with $CuSO_4$. The contents of the tube were shaken gently to elute the color for the quantitative determination. The intensity of the color was determined at 540 mμ wavelength using the Beckman Model DU-spectrophotometer. The concentration of an individual acid was then estimated from the optical density measurements, and with a standard calibration curve, measurements were prepared for each of the known amino acids.

In the case of proline, the ninhydrin stained band was yellow. It was effectively extracted with 75% ethanol only, as the extraction with 75% ethanol saturated with $CuSO_4$ was

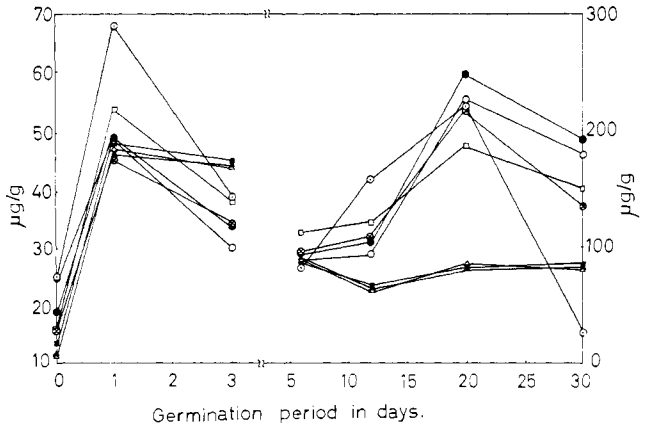

Figure 1. Variation of aspartic acid during germination of γ irradiated groundnut

○ Control light ⊙ Control dark ● 10 Kr
⊗ 30 Kr □ 50 Kr ■ 70 Kr △ 90 Kr ▲ 120 Kr

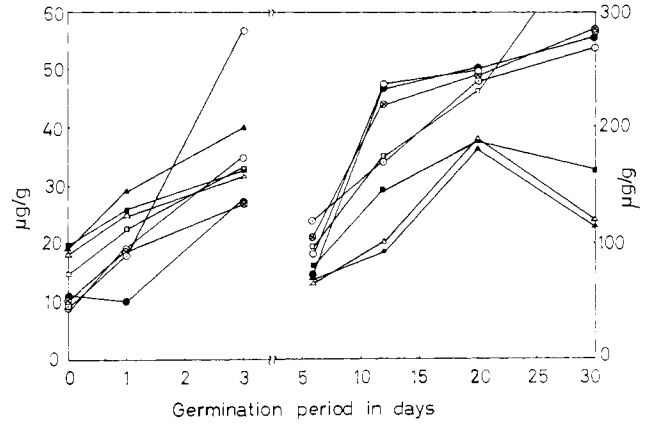

Figure 2. Variation of asparagine during germination of γ-irradiated groundnut. See Legend Figure 1

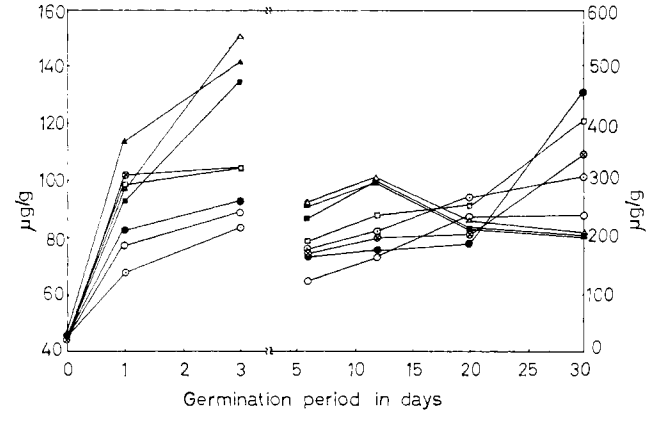

Figure 3. Variation of glutamic acid during germination of γ-irradiated groundnut. See Legend Figure 1

poor. The absorbance measurements were carried out at 450 mμ for proline. Its concentration was estimated from the standard calibration curve prepared in the same manner.

RESULTS AND DISCUSSION

The amount of free amino acids estimated from the two sets of two duplications using the method of circular paper chromatography indicated a relative error of 8 to 10% among the results. Some of the results are plotted in the Figures 1–9.

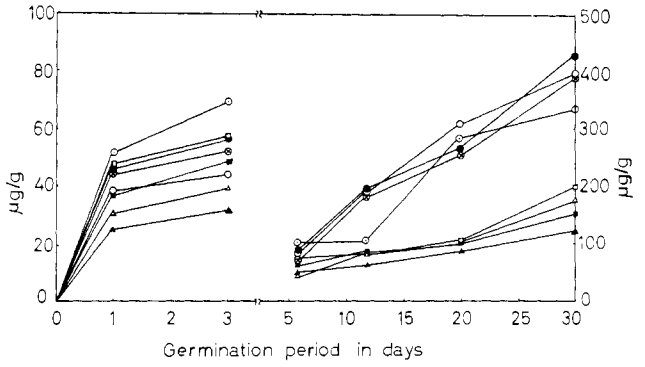

Figure 4. Variation of glutamine during germination of γ-irradiated groundnut. See Legend Figure 1

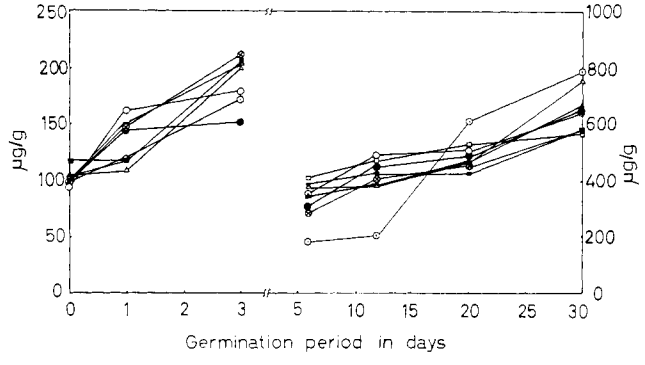

Figure 5. Variation of arginine during germination of γ-irradiated groundnut. See Legend Figure 1

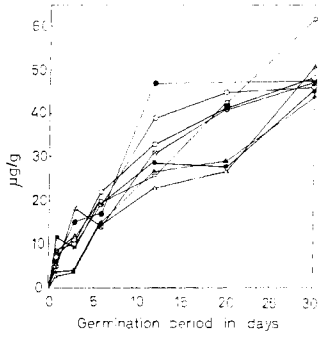

Figure 6. Variation of alanine during germination of γ-irradiated groundnut. See Legend Figure 1

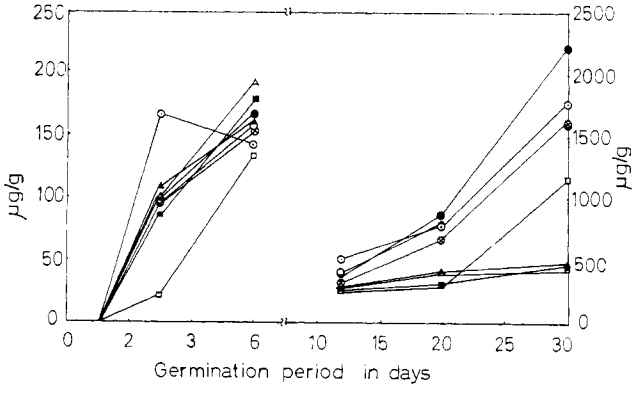

Figure 7. Variation of glycine during germination of γ-irradiated groundnut. See Legend Figure 1

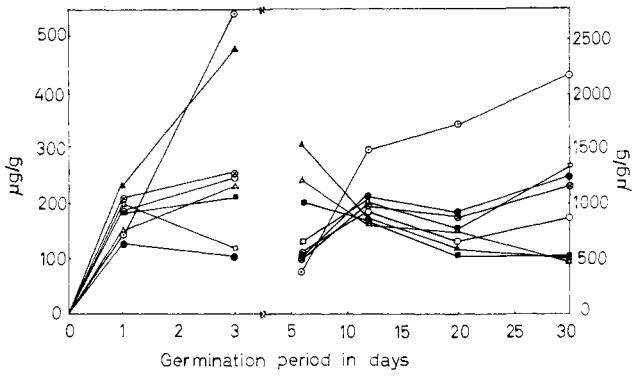

Figure 8. Variation of tyrosine during germination of γ-irradiated groundnut. See Legend Figure 1

In the present variety of groundnut, only four free amino acids (aspartic, asparagine, glutamic, and arginine) were present in estimable amounts; alanine, serine, glycine, and tyrosine were found in traces. Among these free amino acids present in the ungerminated seeds, the amount of arginine was largest, followed by glutamic, aspartic, and asparagine. After 24 hr of germination of the control and the irradiated seeds, the amounts of these acids increased and a few other amino acids also appeared. Table I shows the occurrence of free amino acids with the period of germination. On the third day of germination, the amount of aspartic decreased in all the treatments. This effect was less pronounced for the seeds exposed to 70 to 120 kR dosage levels. It was again found to increase with the period of germination. After 6 days of germination, almost all amino acids were in estimate amounts.

The amounts of free amino acids are found to increase mostly with the period of germination. The increase in the case of aspartic acid is not appreciable up to the twelfth day of germination of the control, and the 10, 30, and 50 kR treated seeds. However, asparagine increased considerably after the sixth day of germination. There was sufficient growth of control seedlings and the seeds exposed to 10 to 50 kR dosage levels. Because of the growth in natural light, the process of photosynthesis may provide carbon precursors necessary for the increase of amides and, as a result, in asparagine. These observations are in agreement with those of Boulter and Barber (1963) on amino acid metabolism during germination of *Vicia faba* L. The growth of seeds irradiated to higher dosage levels is inhibited (Patel, 1965). The asparagine content of these seeds increased during germination, but it was comparatively less than that of the control seeds and the seeds exposed to 10 to 50 kR dosage levels. At the end of 30 days of germination, the asparagine content of the control seeds and the seeds exposed to 10 to 50 kR dosage levels was higher. The large increase in asparagine over aspartic may result from

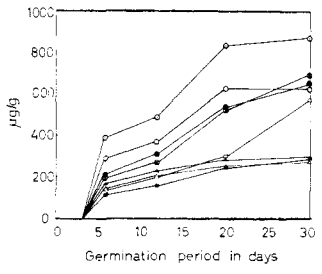

Figure 9. Variation of phenylalanine during germination of γ-irradiated groundnut. See Legend Figure 1

Table I. Occurrence of Free Amino Acids in Groundnut

Sr. No.		Period of Germination (Days) Control Seeds[a]			
		0	1	3	6
1.	Arginine	E	E	E	E
2.	Glutamic	E	E	E	E
3.	Aspartic	E	E	E	E
4.	Asparagine	E	E	E	E
5.	Glutamine	...	E	E	E
6.	Tyrosine	T	E	E	E
7.	Alanine	T	E	E	E
8.	Serine	T	T	E	E
9.	Glycine	T	T	E	E
10.	Methionine	...	T	T	E
11.	Phenylalanine	...	T	T	E
12.	Valine	...	T	T	E
13.	Histidine	...	...	E	E
14.	Threonine	...	...	...	T
15.	Proline	...	...	E	E
16.	Lysine	...	...	...	E
17.	Cysteine	...	...	...	T
18.	Leucine	...	...	...	...
19.	Isoleucine	...	...	...	T

[a] E = estimable amount; T = traces.

the carbohydrate metabolism, as suggested by Prianishikov (1922) and Smirnov (1923). The former pointed out that barley seedlings cut off from the reserve carbohydrate by removing the endosperm produced less amide than usual. The latter noted that the seedlings supplied with an external source of carbohydrate synthesized asparagine in dark. In the present work, carbohydrates of the seedlings increased during germination (Patel, 1965).

There was a regular increase in the amount of glutamic acid in the seedlings of the control and 10 to 50 kR dosage levels. The seeds treated with 70 to 120 kR dosage levels on germination attained maximum glutamic acid content on the twelfth day, and thereafter it decreased in these seedlings. The large increase in the glutamic acid of the seedlings of the control and the irradiated seeds may be due to transamination reactions. Fowden (1953) and Fowden and Webb (1955) reported that with the onset of respiratory activity, groundnut seedlings contained transaminase enzyme capable of forming glutamic acid, alanine, or aspartic acid. Seeds exposed to 70 to 120 kR dosage levels had comparatively slow increase of glutamic acid up to the twelfth day of germination and then there was a decline. Initial increase of the glutamic acid in these seeds may be attributed to the interconversion of amino acids, but lack of growth during the later period of germination ultimately leading to the absence of photosynthesis may inhibit its formation. That is why these seedlings show a decline in glutamic acid content after reaching a maximum value.

There was also a rapid increase of glutamine contents of the seedlings after 12 days of germination of both control and the seeds exposed to 10 and 30 kR dosage levels. This might be ascribed to the cumulative effect of deamination reactions and photosynthesis. Here also the growth of seedlings and photosynthesis seems to have specific effect on the synthesis of glutamine. Thus the seeds exposed to higher dosage levels had low glutamine content on germination.

Alanine, which was in trace amounts in ungerminated seeds, was found to gradually increase during germination. The biosynthesis of alanine may arise from transamination reaction of glutamic acid. The corresponding increase of the other free amino acids (glycine, serine, threonine, tyrosine,

histidine, methionine, phenylalanine, and valine) might be attributed either to the breakdown of carbohydrate and reserve protein of the seeds or to the nitrogen metabolism during germination. The pentose pathway of carbohydrate breakdown has been reported (Rabson and Tolbert, 1957) to be associated with the synthesis of some amino acids. The biosynthesis of tyrosine in the early stage and that of phenylalanine in the later stage (6 days later) of germination might be taking place in the groundnut, according to the mechanism elucidated by Davis (1955).

In general, the variation in the amounts of free amino acids in the seedlings of control and irradiated seeds suggest that the interconversion and biosynthesis are affected by the previous irradiation of seeds. The radiation dose of 10 kR stimulates the synthesis of most of the amino acids. The amounts of glutamic, arginine, serine, glycine, threonine, tyrosine, histidine, and phenylalanine of the control seeds grown in the darkness were higher than those of the control seeds grown in light. Photosynthesis may not be the only factor affecting the increase of some amino acids. Other processes are also operative in the biosynthesis of them.

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