GROWTH AND MINERAL ABSORPTION OF TRIGONELLA FOENUM-GRAECUM, L.

SEEDLINGS FROM SEEDS WASHED FOLLOWING GAMMA IRRADIATION

A DISSERTATION 4161

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CHAPTER I

INTRODUCTION

More than half a century has passed since the first investigations of radiation effects on plants were begun. Since then, a great deal has been learned about ionizing radiations and their deleterious effects on various organisms. It is now known that species differ in their tolerances to radiation. Certain criteria have been established to evaluate radiosensitivity in plants such as the morphological appearance of the plant. Many investigators have reported stimulating effects when low doses of radiation are used.

Morphological changes which accompany growth inhibition, abnormal shapes and sizes of leaves, reduced development of reproductive structures, may not be solely due to genetic alterations or failure in DNA synthesis. These radiation-induced changes may be influenced by the plant's general metabolism and its inability to absorb and/or utilize various nutrients (Gunckel and Sparrow, 1961). They also observed that anatomical changes induced by radiation are often accompanied by alteration in physiological processes. These changes can cause a plant to be unable to absorb or utilize certain essential nutrients and deficiencies of these elements may be seen as modifications in normal growth or appearance of the plants.

The literature is scant about the effect of radiation on mineral absorption. This investigation will be concerned with the absorption of nitrogen and phosphorus from intact Fenogreek bean seedlings.

Another aspect of this study is to investigate the effect of post-irradiation washing of seeds on radiation damage and mineral uptake. Conger et al. (1968) report a new class of radiation damage which appears independent of oxygen and long-lived radicals. This radiation-induced damage was eliminated if seeds were hydrated anaerobically at 0°C for 18 hours following irradiation. It was suggested that the damage may be due to some unstable chemical species which may be removed by the addition of water to the system. With this information, this study will investigate growth and mineral uptake of seedlings from Fenogreek bean seeds washed following gamma irradiation.

CHAPTER II

REVIEW OF LITERATURE

The effects of radiation on plant growth have been investigated for more than fifty years. The effects are largely deleterious and at high doses the effect is usually lethal, although different species of plants vary in their sensitivity to radiation (Sax, 1955).

Species differences in response to radiation have been noted by Sparrow and Christensen (1953). Tradescantia and Lilium show a mild effect at a dose rate of 20R/day while Brassica and Gladiolus show no definite effect at doses lower than 1400 and 4100R/day. There is little doubt that a large number of factors operate to determine the radio-sensitivity of a given species. Changes in auxin and ascorbic acid levels in irradiated plants indicate that these substances may be involved in determining radiosensitivity. Sparrow and Christensen (1953) state that plants such as Tradescantia, Lilium and Vicia, which all have large chromosomes, may have a higher sensitivity to chronic gamma radiation than do plants with small chromosomes.

Differences in response to gamma radiation were also reported by Saric et al. (1961), from experiments on varieties of wheat seeds. It was found that the percentage of germination decreased with increase in dose; also, the height and weight of seedlings were found to decrease. Reactions of varieties towards the same dose were different. Small doses up to 2000 R had a stimulant effect in comparison with the controls. In some varieties 1500 R and 2500 R produced stimulating effects on seedling height but 10,000 R and higher produced deleterious effects.

Many investigators have also found stimulating effects produced by low doses of radiation. Early investigations by Shull and Mitchell (1933), using low doses of x-ray on seeds of wheat, corn, oats, and sunflower, indicated that plant growth was stimulated. Metallic shields were used to filter the rays and doses between 100-200 R were given. Optimum growth occurred with about 115 R units.

As stated by Sax (1955), Johnson, in 1936, showed stimulating effects of low doses of x-rays resulting in taller, more-branched plants with greater average dry weight. In 1932, Breslauets claimed the yield of rye doubled by irradiating the seeds. In that same year Shull and Mitchell obtained a patent covering the use of shielded x-irradiation to increase crop yields. In 1941, Wort found that wheat exposed to low doses of x-ray produced larger plants than did the controls. Sparrow and Christensen (1953) found evidence of the stimulating effect on growth of <u>Anthirrhinum</u> plants when subjected to moderate intensities of the gamma-field at the Brookhaven laboratories. In experimentation on yield of Katahdin potatoes after exposure of the tubers to varied doses of gamma radiation, Sparrow and Christensen (1950) found a significant trend toward better germination when low doses of gamma radiation were used. Similar results were reported by Fischnich et al. (1961) on potato tubers exposed to x-rays and gamma rays. Radiation given in a low dose (100 R) was effective in sprout promotion, but inhibition of sprouting of the potato tubers occurred after exposure to higher doses of x-rays and gamma rays (1000-7000 R).

Sparrow and Christensen (1953) suggested that one criterion to evaluate the effect of radiation was the gross morphological appearance of the plant. In general, a mild effect means a slight decrease in height or vigor of the plant. A severe effect indicates a definite, often dramatic deviation from the normal or control plant in size, vigor, and in many cases general morphology. Thus, in most instances, a "severe effect" indicates acute stunting or dwarfing from which the plant may or may not recover.

According to Casarett (1968) one of the most commonly used criterion of radiosensitivity is seedling height at specific times following exposure. The highest exposures seem to produce the least growth. Some investigators believe the reduction in seedling height following exposure to ionizing radiation may not be solely due to failure in

DNA synthesis but may be influenced by the cell's general metabolism and its failure to utilize various nutrients (Gunckel and Sparrow, 1961).

Sparrow (1951) reported that low doses of gamma radiation were sufficient to produce one or more morphological responses. These included, in addition to growth inhibition, some unusual shapes and sizes of leaves and of reproductive structures. Hypertrophic growth in certain structures was frequently observed. In some species, tumor-like growth as well as somatic mutations were observed. Doses which produced marked morphological changes also produced a considerable amount of chromosomal aberrations. However, since recovery to a normal growth pattern occurred in many plants which had previously shown severe radiation damage, it would seem that most of the morphological aberrations were not of genetic nature. It is considered that they were probably the result of radiation-induced physiological changes although it is possible that they were somewhat related to chromosomal aberrations (Sparrow, 1951).

The subsequent changes observed in plant tissues as the result of radiation may be due to several interrelated facets. According to Lott and Wall (1968), some investigators explain these alterations on the basis of anatomical changes in the various membranes involved. Low doses of radiation may effect the transport mechanisms, whereas higher doses may bring about physical destruction of tissues.

Some attribute the main radiation effect to growth depression and not to permeability changes (Lott and Wall, 1968). Based on their investigations, Lott and Wall believe radiation produces an effect on metabolic transport mechanisms. The changes may be due to a breakdown in the anatomical integrity of the cellular structures.

Mineral Absorption

As reported by Hackett (1966), Gregory in 1937 found that a balanced nutrient supply produced a linear relationship between dry weight and nutrient uptake. In the absence or presence of certain minerals, or in the case of the failure of a plant to utilize particular minerals, the metabolic machinery may be altered (Steward, 1963).

The essentiality of an element can be determined by several factors as withholding or reducing an element to determine if normal growth is effected, if any deficiency diseases are produced, and if certain biochemical reactions are altered. Mineral absorption may be affected by the metabolic activity of the absorbing cells, concentration of ions in the external solution, cell permeability, interionic effects of one ion on another, and the kind and nature of the ions (Terry and Ward, 1959). However, in reports by Humphries (1951), and Russell and Martin (1952), it was found that the effect of one element upon the uptake of another is virtually independent of other ions.

Smith (1962) states that through unknown mechanisms plants have the capacity of selective absorption. This may vary both qualitatively and quantitatively with the species. The physiological age of the plant tissue is probably the most important factor affecting the mineral composition. Each element has a characteristic pattern that accompanies the aging of tissues. Concentrations of nitrogen, phosphorus, and potassium are greater in young tissues than in old, while the reverse tends to be true for some of the other elements.

Redistribution of elements within the plant is a continual process. In many plants new growth is solely dependent on the use of stored minerals. Some leaves may lose 25 to 30% of their nitrogen as the element moves directly into new growth. Younger tissue has priority on newly absorbed nitrogen (Smith, 1962).

It is concluded that the hypothesis of a complex Donnan equilibrium system can account for most of the observations on the initial absorption of ions by plant cells, whereas a process dependent on respiration must be postulated to explain the subsequent irreversible absorption or accumulation, probably into the vacuoles. The initial uptake of anions is much less than that of cations which is consistent with a Donnan equilibrium. The cation exchange and the apparent free space would depend on any metabolic changes in the cytoplasm which influence the concentration of immobile ions (Hope and Robertson, 1956).

There is considerable evidence that the tonoplast is a high resistance boundary to the movement of free ions and the growth of the celi to a predictable shape is indicative of a spatial organization of molecules and of charged sites. Evidence indicates that the turnover of charged sites on macromolecules is a general characteristic of metabolic processes. There is the possibility that active transport is a consequence of the spatial orientation of sites at which metabolic reactions occur on macromolecules in the cytoplasm (Middleton and Russell, 1958).

Nitrogen in Plants

Nitrogen is the most important element taken in by plant roots. It is a macronutrient whose absorption is markedly affected by a combination of factors in the metabolic processes of the cell. Nitrogen enters into the structure of chlorophyll, proteins, nucleic acids, and co-enzymes (Robertson, 1958).

It is very hard, if not impossible, to distinguish between nitrogenous compounds synthesized in situ and those compounds in transit from areas of synthesis in the roots and leaves. As reported by Sawnson (1957), Hay et al., in 1953, concluded that the above-ground parts of corn plants contribute 60% of the total nitrogen in the mature grain and 40% was contributed directly by the roots. It was believed that nitrates were converted into protein-like substances in the leaves and translocated to the grain.

According to Sawnson (1957), this statement of the form of translocated nitrogenous compounds is in contrast to that of Engard, who in 1940 concluded that in the raspberry there was no definite longitudinal translocation of any organic nitrogen compounds either upward or downward in the bark. The nitrogen distribution which does occur is in the form of nitrates, principally in the xylem.

Sawnson (1957) reported chromatographic analysis of phloem sap indicated a relatively low concentration of nitrogenous compounds. It is not known whether the low concentration of nitrogenous compounds found in the phloem exudate is evidence that there is not a valid sample of the nitrogenous translocational materials, or whether the low concentration is compensated by a higher translocation rate, or whether the major portion of the organic nitrogen compounds are synthesized in the fruit in situ from xylem-supplied mineral salts and phloem-supplied sucrose.

Investigators such as Breon and Gillam (1944) have suggested that the process of nitrogen assimilation in the plant passes through a step involving the reduction of nitrates to nitrites and finally to nitrogen carriers which function in the synthesis of amino acids and proteins. Breon and Gillam (1944) describe the theory, advanced in 1924 by Eckerson, which suggested that the reduction process is governed by a reductase which catalyzes the reduction of nitrates to nitrites if certain necessary conditions are met, such as the presence of phosphorus, potassium and calcium.

Mineral deficiencies are recognized by various physical changes in leaf coloration and size, plant height, etc. (Aslander, 1958). In plant studies by Nightingale (1948), it was indicated that environmental influences such as low temperature or high salt concentration in the substrate, may lead to carbohydrate accumulation and deficiency of nitrogen. Excessive nitrogen uptake may cause increase in the number and the size of leaf cells (Devlin, 1967).

Phosphorus in Plants

Phosphorus is continuously circulating up and down the plant. Some studies show that phosphorus does not move laterally in the plant (Smith, 1962). The most likely path of phosphate movement from plant roots is in the transpiration stream, while lateral movement is thought to be regulated by the cambium which is between the xylem and phloem cells. The anionic phosphorus is found in nucleic acids, phospholipids, and coenzymes NAD and ATP (Devlin, 1967). During respiration inorganic phosphate is picked up from the surrounding medium, particularly by the mitochondria and incorporated into ADP to form ATP (Gilbert, 1953).

Hackett (1966) reported that Gregory, in 1947, found a linear relationship between dry weight and nutrient uptake when barley was given a balanced nutrient supply. Usual symptoms of potassium and phosphorus deficiencies were most marked at the extremes of the potassium/phosphorus ratio.

Vasilev and Rybalka (1959) reported that Kuzin and Strazhevskaia, using Lutescens-t2 wheat, found that the roots of 4-day old seedlings showed no larger depression of phosphorus uptake after irradiation with 3,000, 10,000, 15,000 and 20,000 R than did seedlings receiving 1,000 R doses of x-ray. Vasilev and Rubalka used Moskovka wheat and doses of 1,000, 3,000 and 5,000 R. None of these doses depressed mineral uptake. At the 1,000 R level the irradiated plants absorbed amounts that were equal, within the errors of the experiment, to that of the control group. Small differences were found at the 3,000 and 5,000 R levels, with the irradiated seedlings tending to absorb more than the controls. These doses stimulated the plants slightly, but the effect soon vanished. Winter wheat given doses of 10,000 and 50,000 R caused the plants to take up less mineral than the controls, though the differences in all cases were slight. It would seem that mineral uptake is very insensitive to radiation (Vasilev and Rubalka, 1959).

Results of effects of phosphorus deficiency in Spirodela by Bieleski (1968) demonstrated that growth normally occurs at the expense of external phosphate. During deficiency, growth is limited by the rate at which phosphate can be translocated through the tonoplast and tissues to the growing points. Growth ceases when the supply of non-metabolic phosphate is exhausted.

Studies by Russell and Martin (1952), on the absorption and utilization of phosphorus by young barley plants, revealed that when the external concentration of phosphate is reduced, the proportion of absorbed phosphate found in the plant is markedly reduced. Plants treated with low concentrations of phosphate lose the recently absorbed mineral to the outer medium when transferred to phosphate-free solutions. Plants treated with higher concentrations tend to lose a smaller proportion of the recently absorbed phosphate. Devlin (1967) describes symptoms of phosphate deficiency as premature leaf fall, overall stunting, distorted leaves, reduced vascular tissue, increase in pith cells and fewer xylem and phloem cells which have thin walls.

When there is a phosphorus deficiency during growth, the plant accumulates nitrates in the tissues due to an inability to utilize the nitrates (Breon and Gillam, 1944). This causes the plant to exhibit deficiencies of both phosphorus and nitrogen. Tests with tomato plants, using nitrate as a nitrogen source, exhibited moderate symptoms of phosphate deficiency and showed an accumulation of nitrates in all parts of the plant. According to Breon and Gillam (1944), when plants were given different forms of nitrogen at four levels of phosphorus, there was no better relative growth on any of the levels than the plants utilizing nitrate.

Radiation Effects on Plant Growth

Radiation effects on the physiology of cells may be influenced by a wide range of factors such as: available oxygen, amount of water, metabolites, enzyme inhibitors, pH, and temperature. Studies of glycolysis and respiration have not provided evidence of direct interference by radiation with enzyme activity at doses below 10,000-20,000 R (Kahn and Hanson, 1957). In 1956, Strazhevskaia and Kuzin found that 1,000 R of x-rays upset the metabolism of phosphorus-containing compounds in the roots of wheat (Vasilev and Rybalka, 1959).

As previously stated by Gunckel and Sparrow (1961), anatomical changes induced by exposure to ionizing radiation are often accompanied by alteration in physiological processes essential for normal plant growth. Resulting from these changes and perhaps at times contributing to additional alterations are such factors as the plant's ability to utilize available minerals or the deficiency of certain essential nutrients.

Literature is very scant on references to the affect of washing as it will be used in this study. As previously stated, Conger _et al_. (1968) have presented some background information on which basis post-irradiation washing may result in a beneficial effect on damage induced by radiation.

CHAPTER III

MATERIALS AND METHODS

The material used in this study was the Fenogreek bean--*Trigonella foenum-graecum*. This species has rapid seed germination and fast seedling growth. The seeds germinate well in DiSpo growth pouches and are quite tolerant to irradiation. Seeds were individually selected for uniformity by weight 0.025 ± 0.001 mg. There were six treatments and 200 seeds per treatment. The treatments were as follows:

Control---Seeds were neither irradiated nor washed.

15,000 R--Seeds were exposed to a dose of 15,000 R of gamma rays, but the seeds were not washed.

30,000 R--Seeds were exposed to a dose of 30,000 R of gamma rays, but the seeds were not washed.

Control---Seeds were not irradiated, but the seeds Washed were washed.

15,000 R--Seeds were exposed to a dose of 15,000 R Washed of gamma rays and then the seeds were washed.

30,000 R--Seeds were exposed to a dose of 30,000 R Washed of gamma rays and then the seeds were washed.

The dry seeds, containing 6.9% moisture, were wrapped in one layer of cellophane wrap and placed in a fixed position in the source holder for exposure. Co-60 was the radiation source. The dose rate was 900R/minute.

After the proper time of exposure to radiation the seeds to be washed were placed in running tap water for a period of 24 hours, and then they were placed in DiSpo pouches.* Seeds to be washed were placed in running water at the same time as the unwashed seeds were planted in the wet pouches.

Ten seeds were placed in each DiSpo pouch and five pouches represented five replicates for each treatment. The replicates were arranged in a randomized block design. The experimental unit was a pouch of ten plants. The same arrangement was followed for each harvest. Seedlings were harvested 7, 14, 21, and 28 days from planting.

During growth, the pouches were placed in a growth chamber set at 15°C during an eight hour night and at 25°C for a sixteen hour day. The light intensity was set at 24 kilolux. Equal amounts of water and nutrients were given the plants at specified intervals (Devlin, 1967, Table 27, in the Appendix).

At each harvest two plants from each pouch were measured for length with the measurement being from crown to tip.

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*DiSpo pouches are six and one-half inch square, plastic containers containing a paper wick which forms a trough on which the seeds may be placed.

The two plants were sampled at random. All the plants from each pouch (as one unit) were measured for fresh and dry weights. Plant roots were washed with distilled water and then kept in a drying oven at 70°C for a 24-hour period. The plants were then ground with a Wiley laboratory mill. Mineral determinations for the elements nitrogen and phosphorus were made on the dried samples.

Nitrogen was determined by the Perkin-Elmer Model 240 Elemental Analyzer. Procedures used are listed in the instruction manual of the Elemental Analyzer.

Methods for phosphorus analysis were adapted from methods described by Honda (1956). In preliminary investigations, similar dried plant samples were digested in nitric and perchloric acids as well as in sulfuric acid and hydrogen peroxide. Comparison of the two digestion procedures indicated that the former method gave better reproducibility for phosphorus determinations. The absorbance of the digested plant solutions at 600 mμ was compared with that of a standard series containing 0 to 100 parts per million PO_4. While the color did not develop sufficiently when sulfuric acid and peroxide were used, the material digested in nitric and perchloric acids produced the results shown in Table 26 (Appendix). This indicated that the material digested in nitric acid and perchloric acids was more appropriate for the determination of phosphorus. Phosphorus determinations were made on a Hitachi Perkin-Elmer

Model 139 Spectrophotometer at a wavelength of 600 mμ. Digestion procedures were as follows: add 5 ml of concentrated nitric and 1 ml 70% perchloric acids to 0.10 g. of dried plant material, then heat in a boiling water bath for 30 min or until reduced to one half volume. The digest was then brought up to a volume of 25 ml with distilled water and then read on the spectrophotometer.

CHAPTER IV

RESULTS

Rate of Growth

Seedling Heights

The heights of Fenogreek seedlings at various ages following treatments of radiation and washing are presented in Table 1. The figures in the last two columns represent the least significant difference in height between two treatment means within the same age level. It is calculated from values of studentized range at $K_{6,30}$ (Goldstein, 1964).

Table 1 indicates that at the age of 7 days, seedling height was not affected by radiation. The washing treatment did reduce seedling height, if the seeds were not irradiated (Table 2).

With one exception, the 14, 21 and 28-day old seedlings given the 30,000 R dose of gamma radiation were reduced in height to a highly significant extent when compared with the effect of the 15,000 R dose (Tables 1, 3, 4, and 5) (Figures 1 and 2).

The interaction of radiation and washing was seen as the seedlings grew older (Tables 1, 4, and 5). The low

TABLE 1--SEEDLING HEIGHT OF FENOGREEK BEANS IN CM
(FIGURES ARE AVERAGES OF 10 SEEDLINGS)

Age of Seedlings, Days	Control		15,000 R		30,000 R		Least Significant Range Between 2 Treatment Means	
	Non-Washed	Washed	Non-Washed	Washed	Non-Washed	Washed	At 5%	At 1%
7	3.8	3.2	3.9	3.5	3.6	3.3	0.6	0.7
14	6.0	5.7	6.1	5.7	4.2	4.8	0.7	0.9
21	10.4	10.0	7.9	9.3	6.0	6.1	1.1	1.3
28	15.0	13.6	9.3	13.7	7.6	7.9	3.0	3.6

TABLE 2--ANALYSIS OF VARIANCE OF SEEDLING HEIGHT OF 7-DAY OLD FENOGREEK BEANS

Source	d.f.	S.S.	M.S.	F Value
Total	59	19.64		
Replicate	4	2.38	0.595	3.63*
Treatment	5	4.04	0.808	4.93**
Rad. Effect	2	0.95	0.475	2.90
Rad. v. Non-Rad.	1	0.02	0.022	0.13
Low Rad. v. High Rad.	1	0.93	0.928	5.66*
Wash Effect	1	2.77	2.773	16.90**
Rad. X Wash.	2	0.32	0.160	0.98
Treatment X Replicate	20	8.29	0.415	2.53*
Error	30	4.93	0.164	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 3--ANALYSIS OF VARIANCE OF SEEDLING HEIGHT OF 14-DAY OLD FENOGREEK BEANS

Source	d.f.	S.S.	M.S.	F Value
Total	59	54.99		
Replicate	4	1.75	0.438	1.55
Treatment	5	27.69	5.540	19.58*
Rad. Effect	2	24.06	12.031	42.51*
Rad. v. Non-Rad.	1	5.29	5.29	18.69*
Low Rad. v. High Rad.	1	18.77	18.77	66.32*
Wash Effect	1	0.014	0.014	0.05
Rad. X Wash.	2	3.62	1.808	6.38*
Treatment X Replicate	20	17.06	0.853	3.01*
Error	30	8.49	0.283	

*Significant at the 1% level.

TABLE 4--ANALYSIS OF VARIANCE OF SEEDLING HEIGHT OF 21-DAY OLD FENOGREEK BEANS

Source	d.f.	S.S.	M S.	F Value
Total	59	285.82		
Replicate	4	2.63	0.66	1.06
Treatment	5	184.77	36.95	59.60*
Rad. Effect	2	175.04	87.52	141.16*
Rad. v. Non-Rad.	1	111.48	111.48	179.81*
Low Rad. v. High Rad.	1	63.56	63.56	102.52*
Wash Effect	1	1.98	1.98	3.19
Rad. X Wash.	2	7.75	3.86	6.22*
Treatment X Replicate	20	79.97	4.00	6.45*
Error	30	1845	0.62	

*Significant at the 1% level.

TABLE 5--ANALYSIS OF VARIANCE OF SEEDLING HEIGHT OF 28-DAY OLD FENOGREEK BEANS

Source	d.f.	S.S.	M.S.	F Value
Total	59	839.04		
Replicate	4	22.42	5.61	
Treatment	5	542.84	108.56	1.19
Rad. Effect	2	436.17	218.08	46.40*
Rad. v. Non-Rad.	1	293.28	293.28	62.40*
Low Rad. v. High Rad.	1	142.89	142.89	30.40*
Wash Effect	1	17.28	17.28	3.68
Rad. X Wash.	2	89.39	44.70	9.51*
Treatment X Replicate	20	132.86	6.64	1.41
Error	30	140.92	4.70	

*Significant at the 1% level.

Figures 1 and 2--Seedling heights in cm. at various ages with indicated treatments. Vertical lines on the abscissa indicate the least significant difference between two treatment means at the 5% level.

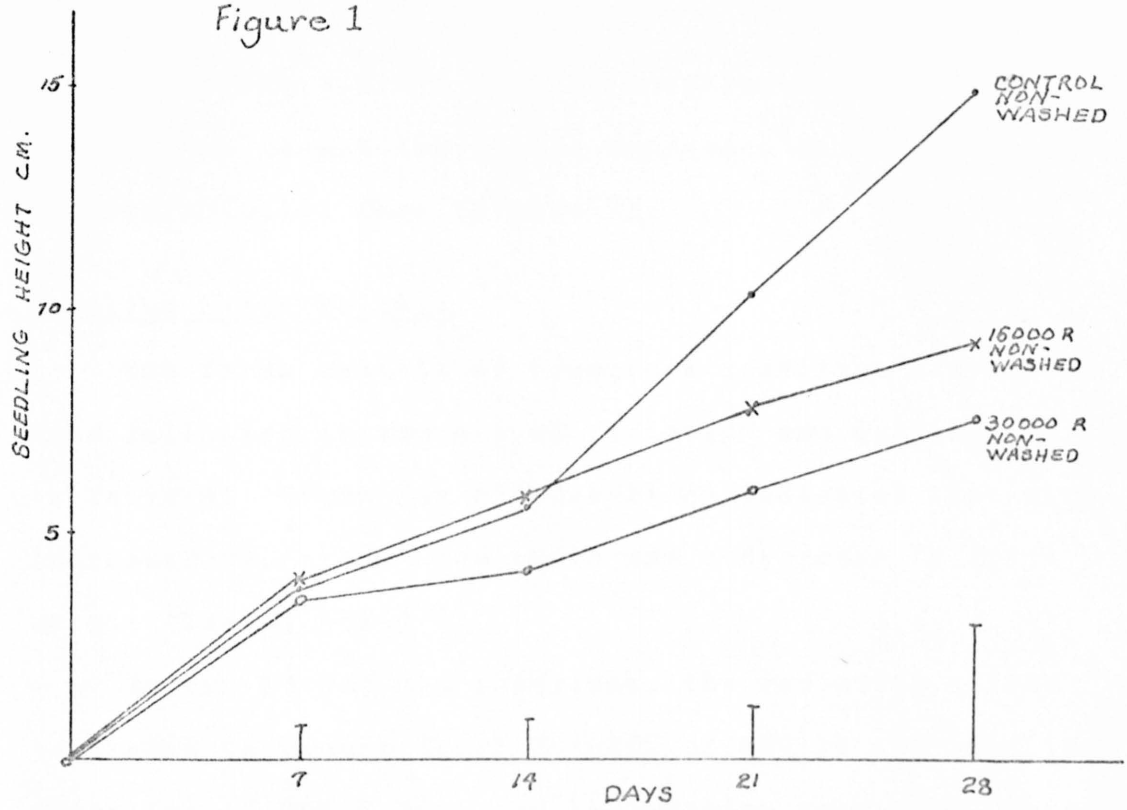

Figure 1
SEEDLING HEIGHT C.M.
15
10
5
7
14
21
28
DAYS
CONTROL NON-WASHED
16000 R NON-WASHED
30000 R NON-WASHED

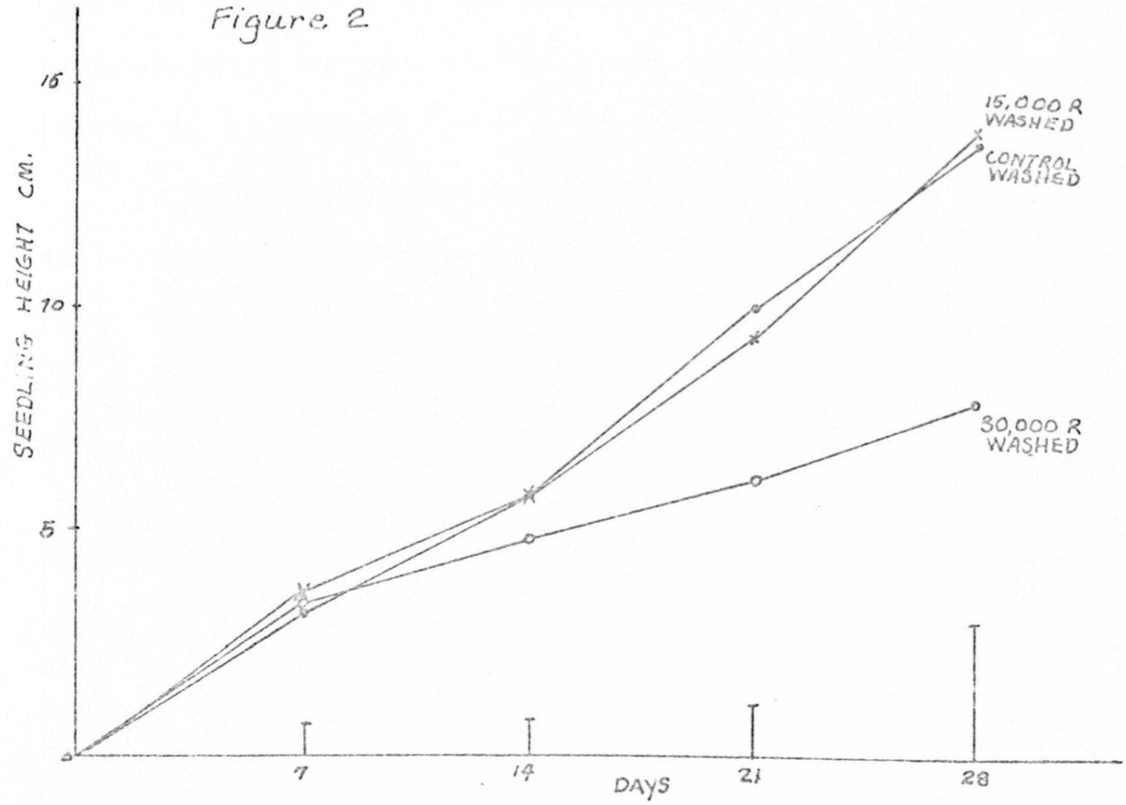

Figure 2
SEEDLING HEIGHT C.M.
15
10
5
0
7
14
21
28
DAYS
15,000 R WASHED
CONTROL WASHED
30,000 R WASHED

radiation dose and the post-irradiation washing effect appeared to reduce the damage expressed as a result of the higher radiation dose (Figure 2).

Seedling Fresh Weights

The fresh weights of Fenogreek seedlings at various ages following treatments of radiation and washing are shown in Table 6. Seven day old seedlings indicated that with increased radiation dose there was a decrease in fresh weight (Tables 6 and 7).

In the 14-day old seedlings, the radiation effect continued to reduce fresh weight, except in the seedlings given the 15,000 R dose and the washing treatment, where there was a slight increase in fresh weight (Tables 6 and 8). There is more than a 95% probability, however, that washing reduced fresh weight if the seeds were not irradiated (Table 6, line 2).

In the older seedlings, the high radiation dose effect noticeably reduced fresh weight (Tables 6, 9, and 10) (Figures 3 and 4). The 15,000 R dose appeared to stimulate plant growth, especially in the seeds that were washed (Table 6) (Figures 3 and 4).

Seedling Dry Weights

Dry weights of the 7-day old seedlings were reduced by both levels of radiation. This reduction was greater, however, in the seedlings receiving the higher dose of

TABLE 6--FRESH WEIGHT OF FENOGREEK BEAN SEEDLINGS IN GRAMS (FIGURES ARE AVERAGES OF 40 SEEDLINGS)

Age of Seedlings, Days	Control		15,000 R		30,000 R		Least Significant Range Between 2 Treatment Means	
	Non-Washed	Washed	Non-Washed	Washed	Non-Washed	Washed	At 5%	At 1%
7	0.1785	0.1611	0.1492	0.1519	0.1412	0.1280	0.0356	0.0441
14	0.4349	0.3225	0.3352	0.3649	0.3366	0.2664	0.0939	0.1163
21	0.6875	0.5245	0.5740	0.4377	0.4450	0.2940	0.1730	0.2142
28	0.7104	0.7090	0.7684	0.8545	0.5543	0.4088	0.2130	0.2638

TABLE 7--ANALYSIS OF VARIANCE OF FRESH WEIGHTS OF 7-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.96125483		
Replicate	4	0.05744498	0.01436124	0.67
Treatment	5	0.47466587	0.09493317	4.42*
Rad. Effect	2	0.39787208	0.19893604	9.27*
Rad. v. Non-Rad.	1	0.31614396	0.31614396	14.73*
Low Rad. v. High Rad.	1	0.08172812	0.08172812	3.81
Wash Effect	1	0.04114403	0.04114403	1.92
Rad. X Wash.	2	0.03564976	0.01782488	0.83
Error	20	0.42914398	0.02145719	

*Significant at the 1% level.

TABLE 8--ANALYSIS OF VARIANCE OF FRESH WEIGHTS OF 14-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	9.64301303		
Replicate	4	1.25384457	0.31346114	2.21
Treatment	5	5.54737555	1.10947511	7.81**
Rad. Effect	2	2.29692225	1.14846112	8.08**
Rad. v. Non-Rad.	1	1.33722038	1.33722038	9.41**
Low Rad. v. High Rad.	1	0.95970187	0.95970187	6.75*
Wash Effect	1	1.45591858	1.45591858	10.25**
Rad. X Wash.	2	1.79453472	0.89726736	6.31**
Error	20	2.84179291	0.14208964	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 9--ANALYSIS OF VARIANCE OF FRESH WEIGHTS OF 21-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	42.5955		
Replicate	4	4.1931	1.048275	2.14
Treatment	5	28.5934	5.718680	11.66*
Rad. Effect	2	14.7073	7.353650	14.99*
Rad. v. Non-Rad.	1	8.7596	8.7596	17.86*
Low Rad. v. High Rad.	1	5.9477	5.9477	12.13*
Wash Effect	1	13.3567	13.3567	27.23*
Rad. X Wash.	2	0.5294	0.2647	0.54
Error	20	9.8091	0.490455	

*Significant at the 1% level.

TABLE 10--ANALYSIS OF VARIANCE OF FRESH WEIGHTS OF 28-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	60.1289		
Replicate	4	4.5737	1.1434	1.55
Treatment	5	40.8438	8.1688	11.10*
Rad. Effect	2	36.1014	18.0507	24.54*
Rad. v. Non-Rad.	1	1.6554	1.6554	2.25
Low Rad. v. High Rad.	1	34.4460	34.4460	46.83*
Wash Effect	1	0.2218	0.2218	0.30
Rad. X Wash.	2	4.5206	2.2603	3.07
Error	20	14.7114	0.735570	

*Significant at the 1% level.

Figures 3 and 4--Seedling fresh weights in grams at various ages with indicated treatments. Vertical lines on the abscissa indicate the least significant difference between two treatment means at the 5% level.

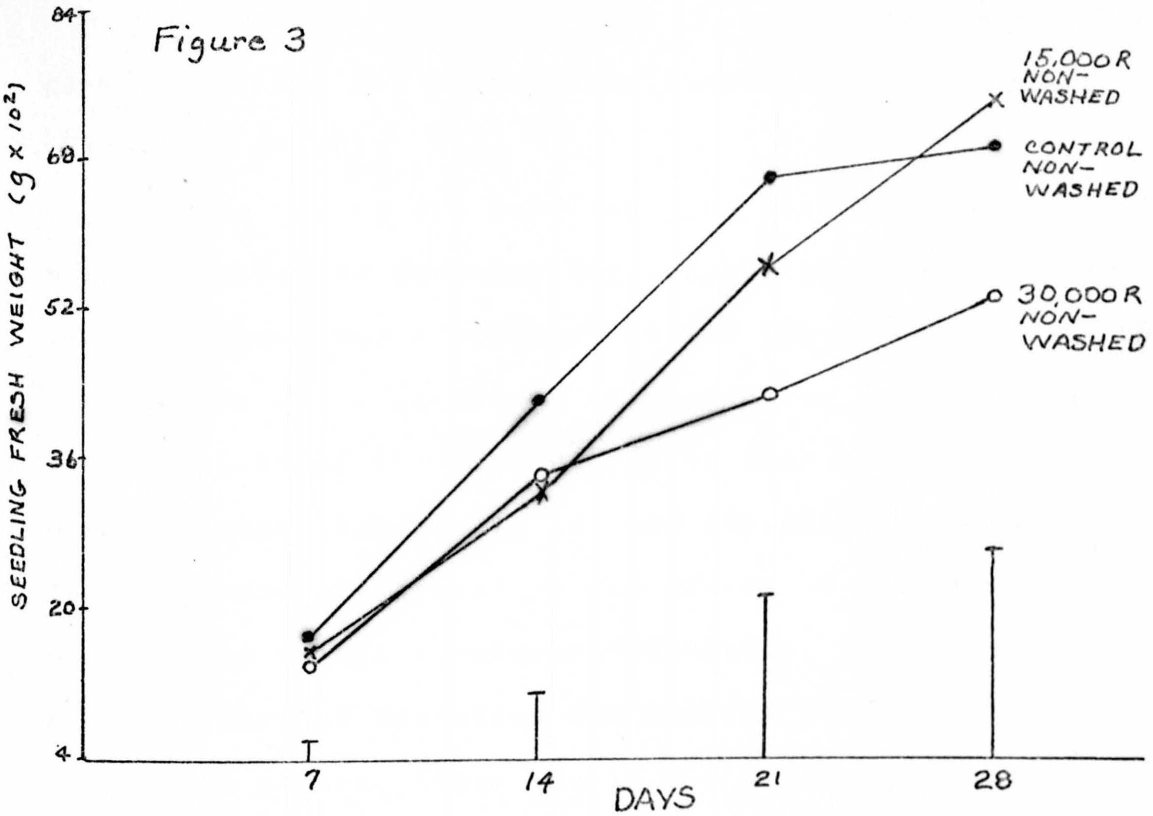
Figure 3
SEEDLING FRESH WEIGHT (g × 10²)
84
68
52
36
20
4
7
14
21
28
DAYS
15,000 R NON-WASHED
CONTROL NON-WASHED
30,000 R NON-WASHED

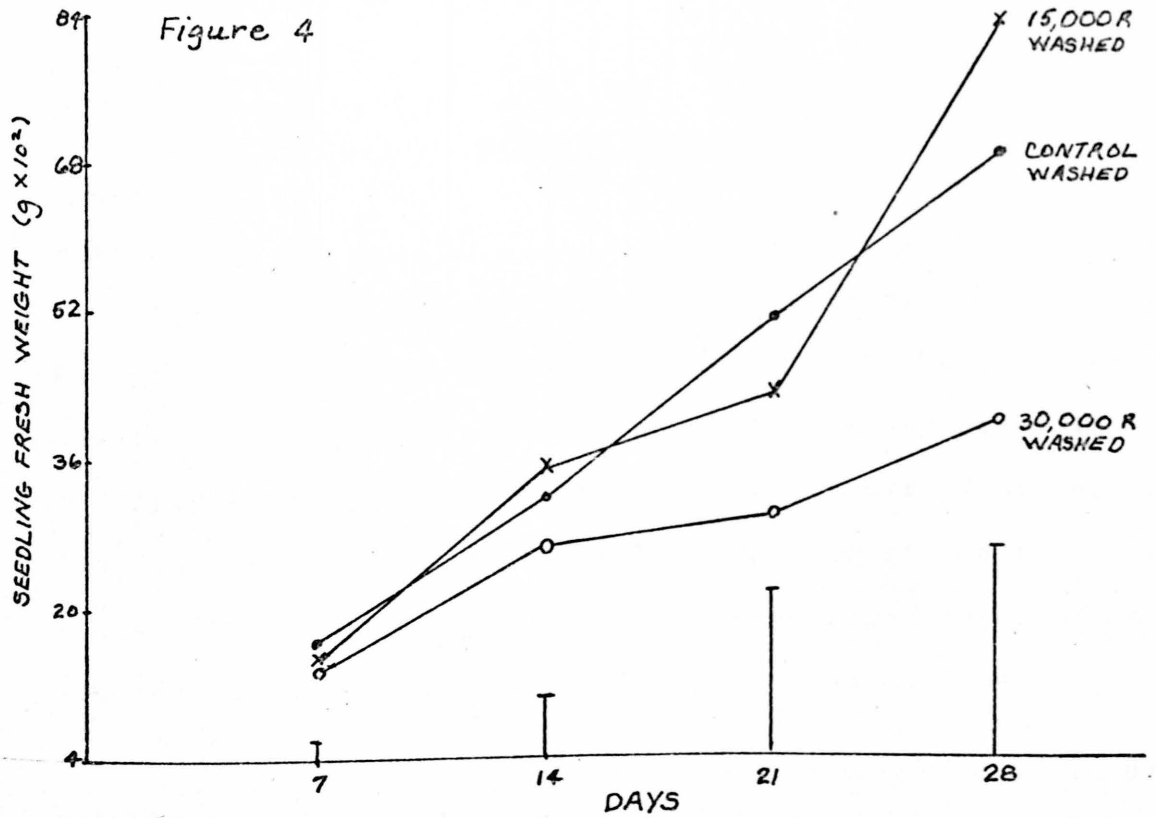
Figure 4
SEEDLING FRESH WEIGHT (g × 10²)
84
68
52
36
20
4
7
14
21
28
DAYS
15,000 R WASHED
CONTROL WASHED
30,000 R WASHED

gamma radiation and the washing treatment (Tables 11 and 12) (Figures 5 and 6).

In the 14-day old seedlings the high dose effect was more effective in reducing dry weight, especially if the seeds had been washed (Tables 11 and 13) (Figures 5 and 6).

In the older seedlings the reduction in dry weights, as the result of the high radiation dose effect, was even more apparent (Tables 11, 14, and 15) (Figures 5 and 6). In the 21-day old seedlings the effect of washing reduced dry weights in all treatments (Table 11). The interaction of the effect of radiation and washing is seen in the 28-day old plants, where the 15,000 R group demonstrated an increase in dry weight as compared with the plants given the high radiation dose and the control group (Table 11) (Figure 6).

Mineral Content*

Nitrogen

The mean percentages of nitrogen content of dry Fenogreek bean seedlings are presented in Table 16. Generally, seedlings at all ages did not show any significant differences in nitrogen content as the result of either seed irradiation or washing (Table 16), that is if two treatment means are compared. The 7-day old seedlings indicated no significant change in nitrogen uptake (Tables 16 and 17).

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*In this study content is expressed as per cent in dry seedlings of Fenogreek beans.

TABLE 11--DRY WEIGHT OF FENOGREEK BEAN SEEDLINGS IN GRAMS
(FIGURES ARE AVERAGES OF 40 SEEDLINGS)

Age of Seedlings, Days	Control		15,000 R		30,000 R		Least Significant Range Between 2 Treatment Means	
	Non-Washed	Washed	Non-Washed	Washed	Non-Washed	Washed	At 5%	At 1%
7	0.0166	0.0149	0.0151	0.0145	0.0151	0.0143	0.0010	0.0012
14	0.0302	0.0226	0.0244	0.0252	0.0256	0.0181	0.0061	0.0076
21	0.0547	0.0411	0.0459	0.0328	0.0332	0.0209	0.0139	0.0172
28	0.0961	0.0691	0.0646	0.0706	0.0521	0.0329	0.0246	0.0304

TABLE 12--ANALYSIS OF VARIANCE OF DRY WEIGHTS OF 7-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.00237663		
Replicate	4	0.00016028	0.00004007	0.66
Treatment	5	0.0010065	0.0002013	3.33*
Rad. Effect	2	0.00037870	0.0001893	3.12
Rad. v. Non-Rad.	1	0.000375	0.000375	6.20*
Low Rad. v. High Rad.	1	0.0000037	0.0000037	0.06
Wash Effect	1	0.0005270	0.0005270	8.71**
Rad. X Wash.	2	0.0001008	0.0000505	0.83
Error	20	0.00120982	0.000060491	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 13--ANALYSIS OF VARIANCE OF DRY WEIGHTS OF 14-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.04184235		
Replicate	4	0.00425106	0.00106276	1.71
Treatment	5	0.02518873	0.00503775	8.12**
Rad. Effect	2	0.00671836	0.00335919	5.42*
Rad. v. Non-Rad.	1	0.00453342	0.00453342	7.31*
Low Rad. v. High Rad.	1	0.00217494	0.00217494	3.51
Wash Effect	1	0.00993720	0.00993720	16.02**
Rad. X Wash.	2	0.00853317	0.00426659	6.88*
Error	20	0.01240256	0.000620128	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 14--ANALYSIS OF VARIANCE OF DRY WEIGHTS OF 21-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.31550656		
Replicate	4	0.02779227	0.00694806	2.12
Treatment	5	0.22223672	0.04444734	13.58*
Rad. Effect	2	0.14134886	0.07067443	21.59*
Rad. v. Non-Rad.	1	0.10055154	0.10055154	30.71*
Low Rad. v. High Rad.	1	0.03079732	0.03079732	9.41*
Wash Effect	1	0.08078565	0.08078565	24.68*
Rad. X Wash.	2	0.00010221	0.00005110	0.02
Error	20	0.06547757	0.00327387	

*Significant at the 1% level.

TABLE 15--ANALYSIS OF VARIANCE OF DRY WEIGHTS OF 28-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.92026166		
Replicate	4	0.03708250	0.00927062	1.05
Treatment	5	0.70723175	0.14144635	16.08**
Rad. Effect	2	0.52506681	0.26253340	29.84**
Rad. v. Non-Rad.	1	0.32432613	0.32432613	36.87**
Low Rad. v. High Rad.	1	0.20074068	0.20074068	22.82**
Wash Effect	1	0.08629338	0.08629338	9.80**
Rad. X Wash.	2	0.09587156	0.04798578	5.45*
Error	20			

*Significant at the 5% level.
**Significant at the 1% level.

Figures 5 and 6--Seedling dry weights in grams at various ages with indicated treatments. Vertical lines on the abscissa indicate the least significant difference between two treatment means at the 5% level.

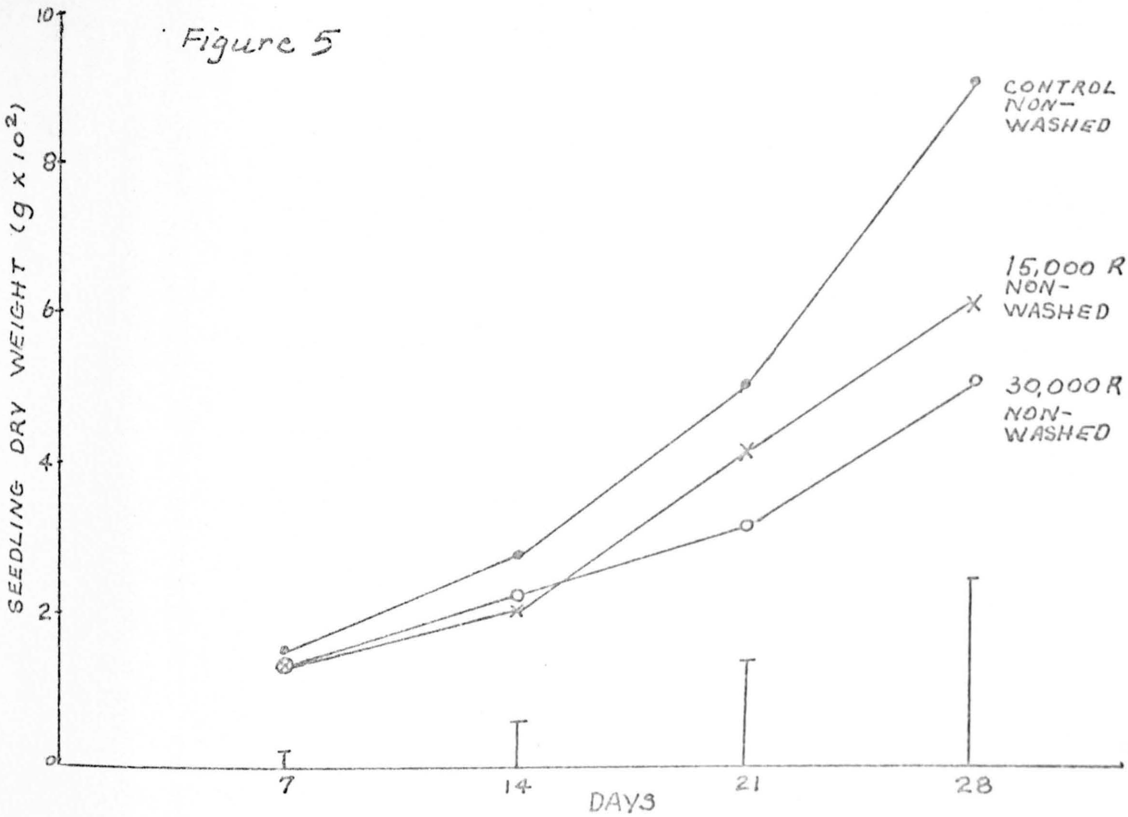
Figure 5
CONTROL NON-WASHED
15,000 R NON-WASHED
30,000 R NON-WASHED
SEEDLING DRY WEIGHT (g x 10²)
10
8
6
4
2
0
7
14
21
28
DAYS

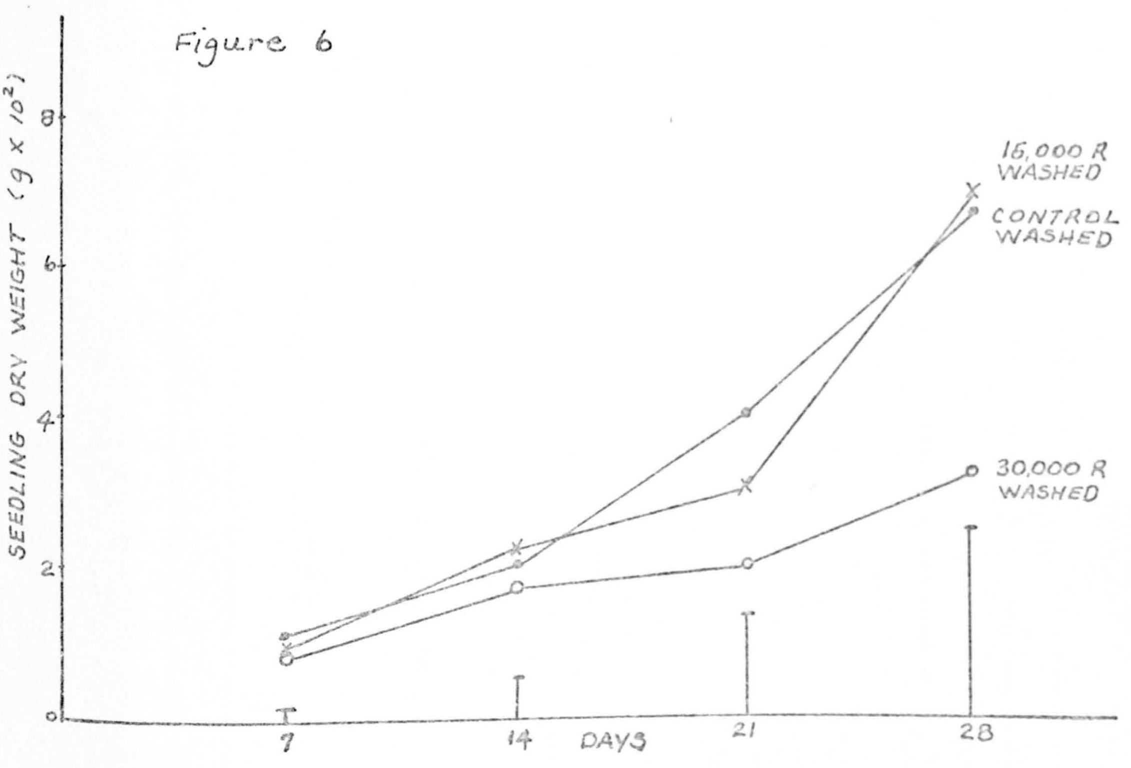
Figure 6
16,000 R WASHED
CONTROL WASHED
30,000 R WASHED
SEEDLING DRY WEIGHT (g x 10²)
8
6
4
2
0
7
14
21
28
DAYS

TABLE 16--PERCENTAGES OF NITROGEN IN DRY SEEDLINGS OF FENOGREEK BEANS (FIGURES ARE AVERAGES OF FIVE REPLICATES)

Age of Seedlings, Days	Control		15,000 R		30,000 R		Least Significant Range Between 2 Treatment Means
	Non-Washed	Washed	Non-Washed	Washed	Non-Washed	Washed	5% level
7	5.47	5.20	5.47	5.66	5.54	5.55	1.068
14	4.57	5.05	4.93	5.44	4.81	5.25	1.001
21	3.93	4.16	3.79	4.35	4.78	4.63	1.202
28	3.12	3.34	3.44	3.62	3.90	4.37	1.113

TABLE 17--ANALYSIS OF VARIANCE OF PER CENT OF NITROGEN IN 7-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	9.9700		
Replicate	4	3.5200	0.8800	3.01*
Treatment	5	0.6000	0.1200	0.41
Rad. Effect	2	0.3356	0.1678	0.57
Rad. v. Non-Rad.	1	0.3320	0.3320	1.14
Low Rad. v. High Rad.	1	0.0036	0.0036	0.12
Wash Effect	1	0.0029	0.0029	0.01
Rad. X Wash.	2	0.2615	0.1308	0.45
Error	20	5.8500	0.2925	

*Significant at the 5% level.

The breakdown of treatments sums of squares reveals some interesting results. For example, the 14-day old seedlings contained a high percentage of nitrogen as a result of the washing effect. The washed seeds produced seedlings which exhibited systematically greater nitrogen content (Table 16 line 2 and Table 18 line 7).

In the older seedlings an increase in radiation dose caused an increase in the percentage of nitrogen (Tables 16, 19 and 20). In other words, at the 21st and 28th days nitrogen percentages were higher in seedlings as a result of high radiation dose as compared to either low radiation or no radiation.

Phosphorus

The mean percentages of phosphorus determined as phosphate content of dry Fenogreek bean seedlings are presented in Table 21. With the exception of the 7-day old seedlings, there was no difference in phosphorus content as a result of the radiation or washing effect, if individual treatment means were compared (Tables 21, 22, 23, 24, and 25). In general the 7-day old seedlings given either the low radiation dose or no radiation demonstrated an increase in phosphorus content which was significant, when compared with those receiving the high radiation dose; however, such a difference was not consistent in subsequent ages.

TABLE 18--ANALYSIS OF VARIANCE OF PER CENT OF NITROGEN IN 14-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	11.4200		
Replicate	4	3.9400	0.9850	3.88*
Treatment	5	2.4100	0.4820	1.90
Rad. Effect	2	0.7159	0.3580	1.41
Rad. v. Non-Rad.	1	0.5926	0.5926	2.34
Low Rad. v. High Rad.	1	0.1233	0.1233	0.49
Wash Effect	1	1.7019	1.7019	6.71*
Rad. X Wash.	2	0.9900	0.4950	1.95
Error	20	5.0700	0.2535	

*Significant at the 5% level.

TABLE 19--ANALYSIS OF VARIANCE OF PER CENT OF NITROGEN IN 21-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S	M.S.	F Value
Total	29	16.6400		
Replicate	4	5.5700	1.3900	3.80*
Treatment	5	3.7600	0.7520	2.05
Rad. Effect	2	2.7990	1.3995	3.82*
Rad. v. Non-Rad.	1	0.7766	0.7766	2.12
Low Rad. v. High Rad.	1	2.0224	2.0224	5.52*
Wash Effect	1	0.3260	0.3260	0.89
Rad. X Wash.	2	0.6350	0.3175	0.87
Error	20	7.3100	0.3660	

*Significant at the 5% level.

TABLE 20--ANALYSIS OF VARIANCE OF PER CENT OF NITROGEN IN 28-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	15.2800		
Replicate	4	3.8800	0.9700	3.02*
Treatment	5	4.9700	0.9970	3.10*
Rad. Effect	2	4.2201	2.1101	6.56**
Rad. v. Non-Rad.	1	2.4081	2.4081	7.49*
Low Rad. v. High Rad.	1	1.8120	1.8120	5.64*
Wash Effect	1	0.6250	0.6250	1.94
Rad. X Wash.	2	0.1249	0.0624	0.19
Error	20	6.4300	0.3215	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 21--PERCENTAGES OF PHOSPHORUS (PO_4) IN DRY SEEDLINGS OF FENOGREEK BEANS (FIGURES ARE AVERAGES OF FIVE REPLICATES)

Age of Seedlings, Days	Control		15,000 R		30,000 R		Least Significant Range Between 2 Treatment Means
	Non-Washed	Washed	Non-Washed	Washed	Non-Washed	Washed	5% level
7	0.737	0.662	0.746	0.788	0.672	0.509	0.223
14	1.112	0.878	0.993	1.093	0.878	0.913	0.355
21	1.170	1.039	1.156	1.046	1.270	1.241	0.209
28	0.904	0.800	0.777	0.946	0.819	1.060	0.462

TABLE 22--ANALYSIS OF VARIANCE OF PER CENT OF PHOSPHORUS IN 7-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.6200		
Replicate	4	0.1100	0.0275	2.20
Treatment	5	0.2600	0.0520	4.16*
Rad. Effect	2	0.1590	0.0795	6.36*
Rad. v. Non-Rad.	1	0.0020	0.0020	0.16
Low Rad. v. High Rad.	1	0.1570	0.1570	12.56*
Wash Effect	1	0.0330	0.0330	2.64
Rad. X Wash.	2	0.0680	0.0340	2.72
Error	20	0.2500	0.0125	

*Significant at the 1% level.

TABLE 23--ANALYSIS OF VARIANCE OF PER CENT OF PHOSPHORUS IN 14-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	1.2000		
Replicate	4	0.3000	0.0750	2.42
Treatment	5	0.2800	0.0560	1.81
Rad. Effect	2	0.1100	0.0550	1.77
Rad. v. Non-Rad.	1	0.0026	0.0026	0.08
Low Rad. v. High Rad.	1	0.1074	0.1074	3.46
Wash Effect	1	0.0082	0.0082	0.26
Rad. X Wash.	2	0.1618	0.0809	2.61
Error	20	0.6200	0.0310	

TABLE 24--ANALYSIS OF VARIANCE OF PER CENT OF PHOSPHORUS IN 21-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	0.9200		
Replicate	4	0.4800	0.1200	10.91**
Treatment	5	0.2300	0.0460	4.18**
Rad. Effect	2	0.1557	0.0778	7.07**
Rad. v. Non-Rad.	1	0.0318	0.0318	2.89
Low Rad. v. High Rad.	1	0.1239	0.1239	11.26**
Wash Effect	1	0.0630	0.0630	5.73*
Rad. X Wash.	2	0.0113	0.0056	0.51
Error	20	0.2200	0.0110	

*Significant at the 5% level.
**Significant at the 1% level.

TABLE 25--ANALYSIS OF VARIANCE OF PER CENT OF PHOSPHORUS IN 28-DAY OLD FENOGREEK BEAN SEEDLINGS

Source	d.f.	S.S.	M.S.	F Value
Total	29	1.3900		
Replicate	4	0.0400	0.0100	0.18
Treatment	5	0.2780	0.0540	1.00
Rad. Effect	2	0.0499	0.0250	0.46
Rad. v. Non-Rad.	1	0.0100	0.0100	0.18
Low Rad. v. High Rad.	1	0.0399	0.0399	0.74
Wash Effect	1	0.0670	0.0670	1.24
Rad. X Wash.	2	0.1531	0.0766	1.41
Error	20	1.0800	0.0540	

In the 21-day old seedlings the radiation effect produced by the high dose was demonstrated by an increase in the content of phosphorus, but this trend was neither true at the 14th nor the 28th days. There appeared to be a reduction in the percentage of phosphorus in all the washed treatments at the age of 21 days (Tables 21, 23, 24, and 25).

CHAPTER V

DISCUSSION

Rate of Growth

The present study on seedling heights reveals that radiation damage may be expressed as a reduction in heights, as seen in the 14, 21 and 28-day old seedlings. This reduction was more severe in seedlings given the higher dose of gamma radiation. Similar results were reported by Sarić _et al_. (1961) in the response of wheat seedlings to gamma radiation. Radiation did not appear to reduce height in the 7-day old seedlings, but since this was later apparent in the subsequent age groups, it could be considered as a delayed expression of radiation-induced injury.

According to Casarett (1968), highest exposures to ionizing radiation produce reduction in growth, a common criterion of radiosensitivity. Sparrow (1951) described the effect of low doses of gamma radiation as the inhibition of growth.

Gunckel and Sparrow (1961) suggested the reduction in height may be partly due to the cell's metabolism and inability to utilize certain nutrients. It would appear that the 30,000 R dose was sufficient to produce height reduction, but not high enough to cause a decrease in mineral uptake. From investigations by Vasilev and Rybalka (1959), using wheat seedlings, low to moderate doses of x-irradiation stimulated phosphorus uptake. Doses of 50,000 R were required to produce even slight decrease in mineral absorption.

Although the 15,000 R caused a reduction in seedling heights, it appeared that washing the seeds immediately after irradiation caused recovery later as seen in the 21 and 28-day old seedlings. This reduction in damage may be the result of the removal of some deleterious factor from the seeds as a result of the washing treatment.

Fresh weights were generally decreased by radiation, with the effect more apparent as the seedlings grew older and as the dose increased. This possibly is the manifestation of altered metabolic activity as the result of radiation. An exception was the increase in fresh weights when the low dose was given and which was even more apparent when the seedlings were from seeds which had been washed. Perhaps the washing treatment removed some materials from the seeds which caused the injury to be reduced.

In this study the changes produced in seedling dry weights generally paralleled the changes demonstrated in seedling heights. One expression of radiation damage may be measured as a reduction in seedling dry weights. This reduction may be evidence of insufficient activity within the seedling.

As reported by Sarić <u>et al</u>. (1961), increase in radiation dose caused decrease in wheat seedling dry weight as well as height. Fang (1969) reported a reduction in dry weights in her research involving gamma-irradiated Fenogreek beans.

The reduction in dry weights in the 21-day old seedlings from washed seeds could perhaps be attributed to the removal by washing of some elements and/or enzymes from the seeds, thus resulting in a delay of activity in the growing seedlings.

The increase in dry weights in the 28-day old seedlings from seeds given the low dose and washing treatments parallels that of the increase in height as well as fresh weights. This could indicate recovery from radiation injury which was not evident in the younger seedlings.

Summarizing the effects of radiation and washing on seedling height, fresh and dry weights, it is found that except for the 7-day old seedlings, radiation effect tended to decrease seedling height, especially as the dose of radiation increased. In the older seedlings the washing treatment appeared to be advantageous in promoting recovery, particularly with low radiation dose.

The fresh weights were generally reduced as the result of the radiation effect, except in the older seedlings that were from washed seeds given the low dose. The dry weights were generally corresponding to heights, and were noticeably reduced as a result of washing effects. This reduction in dry weights may be, in part, the result of larger intercellular spaces in the washed treatments (Freeman, 1970). In her study, which was done on Fenogreek beans, the percentage of intercellular spaces in the leaves of the older seedlings was found to be enhanced by both the effects of radiation and washing.

Mineral Content

Although differences in nitrogen uptake, expressed as per cent of dry Fenogreek seedlings, were not significantly changed by irradiation or washing, the younger seedlings contained higher nitrogen percentages as compared with the older plants. According to Robertson (1958), and Smith (1962), nitrogen appears in higher concentrations in active metabolizing areas of young plants. In these growing regions, non-vacuolated, dividing cells take up quantities of metabolic nitrogen and phosphorus as anions. The older plants usually synthesize carbohydrates faster than nitrogenous compounds, thus resulting in a lower percentage of nitrogen.

The increase in nitrogen content when the 14-day old seedlings were from washed seeds is perhaps a manifestation of their dry weight reduction. As was discussed above, the dry weights were reduced as a result of washing. Furthermore, the tendency of the high dose of radiation to cause loss in dry weights was associated with increase of nitrogen percentages in the 21 and 28-day old seedlings. Vasilev and Rybalka (1959), in studies of the effects of x-irradiation on mineral absorption in wheat seedlings, found that doses of 3,000 and 5,000 R stimulated the plants slightly, but the effect soon vanished. These authors, however, were referring to minerals other than nitrogen, as will be mentioned later. Besides differences in radiosensitivity between wheat and Fenogreek beans, levels of radiation are different. According to researchers such as Shull and Mitchell (1933), and Sparrow and Christensen (1953), low doses of radiation appear to stimulate metabolism and plant growth in general, but higher doses do not. It is suggested, then, that as the seedlings from severely irradiated seeds grew older their dry weights were reduced. At the same time, their nitrogen content increased compared to seedlings from the non-irradiated or lightly irradiated seeds. This could account for higher nitrogen percentages.

Although the phosphorus content in per cent of seedling dry weights, appeared to increase with age at least until the 21st day, there was no particular pattern to indicate radiation or washing effects. Referring to the detailed analysis of variance, however, it may be postulated that a high dose of radiation on seeds may reduce metabolic activity resulting in phosphorus uptake, which is indicated as a lower phosphorus percentage in the 7-day old seedlings from the 30,000 R group as compared to the group receiving the lower radiation dose. At this age, the dry weights of seedlings are not greatly affected by radiation, since it is mostly stored food in the seed.

By the age of 14 days, these seedlings start to show delayed radiation damage, indicated by reduced dry weights, thus increasing their phosphorus content to a level of no significance. At the 21st day dry weight reduction was further manifested, and the corresponding phosphorus content increase became significant. By the 28th day the gap in phosphorus content became smaller indicating no significance, though still high.

Higginbotham and Mika (1954), giving x-irradiation of 3,000 to 100,000 R to discs of potato tubers, found the mineral relations of the tuber tissue was relatively insensitive to x-rays. At doses of 30,000 R, or greater, mineral accumulation varied, but was consistently less than in the controls.

In a report "Mineral Uptake by Plant Roots After X-Irradiation," Vasilev and Rybalka (1959) state that studies of mineral uptake by Kuzin and Strazhevskaia in 1956, using wheat seedlings and x-ray and radioisotope methods, indicated that 1,000 R doses lessened phosphorus uptake in the first few hours after irradiation, but seedlings subsequently recovered and took up materials just as rapidly as did the controls. Doses of 3,000, 10,000, 15,000, and 20,000 R produced no larger depression of phosphorus uptake. These researchers also reported results by Forssberg, in 1943, which demonstrated that radiation did not alter the uptake by roots of oat-seedlings. Summarizing their own investigations, Vasilev and Rybalka state, "Wheat seedlings were given radiation doses of 1000, 2000, 3000, 5000, 10,000 and 50,000 R; it was found that mineral uptake is very insensitive to radiation, and is almost unaffected by the above doses. A slight stimulation was detectable after doses of 3000 and 5000; the effect soon vanished."

Such a statement about mineral uptake may be applicable under their experimental conditions and material. It should be mentioned here that their measurements were made on root tips, where the dry weights are not as important a factor as they are in the case of the whole seedling.

Washing the seeds did not seem to affect phosphorus content of seedlings, though at the 21st day a significant decrease was observed. Unless this age is critical in the washed group with regards to phosphorus uptake, such an observation may be only a chance event.

Reference can be made to the anatomical findings of Freeman (1970), using gamma-irradiated Fenogreek seedlings. There was a highly significant reduction in the diamcter of the xylem cells in the washed controls, as compared to the non-washed controls. Both Smith (1962), and Baker and

Moorby (1969), report that the most likely path of phosphorus movement is up and down in the transpiration stream. If this latter assumption is accepted, it could mean that the seedlings with smaller xylem elements translocate smaller amounts of phosphates proportional to the size of their xylem elements, thus, resulting in a reduced percentage.

In relating the effects of radiation and washing to mineral uptake, it is noticed that mineral uptake of nitrogen and phosphorus is directly affected by radiation damage. High doses of radiation result in a decreased dry weight of seedlings and an increase in both nitrogen and phosphorus percentages. If the assumption is true that phosphorus is translocated mainly in the transpiration stream, then the uptake of phosphorus would be related to the size of the xylem elements. Therefore, it is possible that the reduction in xylem cell size plays a role in restricting phosphorus uptake.

CHAPTER VI

SUMMARY AND CONCLUSIONS

1. This investigation proposed to study: (1) the effect of gamma radiation on seedling height, fresh weight, dry weight, nitrogen and phosphorus content of Fenogreek bean seedlings; (2) the influence of post-irradiation washing of seeds on radiation damage; and (3) the effect of post-irradiation washing of seeds on mineral uptake of nitrogen and phosphorus.

2. Seeds were exposed to two doses of gamma radiation (15,000 R and 30,000 R) from a Co-60 source. Half of the irradiated seeds was planted immediately following irradiation, and the other half was placed under running tap water for a 24-hour period and then placed in DiSpo growth pouches.

3. Seeds of all treatments, arranged in five replicated randomized blocks, were grown in a growth chamber under near optimum conditions. Seedlings were harvested at intervals of 7, 14, 21, and 28 days.

4. The high dose of radiation (30,000 R) decreases seedling height, fresh weight, and dry weight, especially as the seedlings grew older.

5. In the older seedlings the high dose effect was demonstrated by an increase in nitrogen and phosphorus content, apparently as a result of dry weight reduction.

6. The low radiation dose (15,000 R) effect reduced seedling dry weight, but usually the effect was delayed in expression. Generally, the low dose of radiation, when accompanied with the washing treatment, reduced radiation injury so that recovery was indicated by an increase in seedling height.

7. When the seeds were given the low dose of gamma rays and washed, there was generally an increase in nitrogen and phosphorus content in the seedlings.

8. If the non-irradiated seeds were washed, the seedlings generally showed a decrease in height, fresh weight, and dry weight. The decrease was not significant in all instances. However, the washing treatment per se reduced seedling dry weight significantly in every age group.

9. In general, nitrogen was increased, while phosphorus content was decreased in seedlings from non-irradiated, washed seeds.

APPENDIX

TABLE 26--PHOSPHORUS DETERMINATIONS IN DRIED FENOGREEK BEAN SEEDLINGS DIGESTED IN NITRIC-PERCHLORIC ACIDS AND IN SULFURIC ACID-HYDROGEN PEROXIDE

Sample	Nitric-Perchloric Acids %T*	Sulfuric Acid, Hydrogen Peroxide %T	Sample	Nitric-Perchloric Acids %T	Sulfuric Acid, Hydrogen Peroxide %T
A-1	71.5	57.5	B-1	74.5	66.2
A-2	72.2	64.0	B-2	75.0	60.8
A-3	72.2	43.8	B-3	72.0	62.7
A-4	72.5	61.2	B-4	72.0	54.8
A-5	71.0	56.5	B-5	71.8	58.8

*%T refers to transmittance.

TABLE 27--THE COMPOSITION OF NUTRIENT SOLUTION*

Salt	Gram/Liter
KNO_3	0.505
$Ca(NO_3)_2$	0.820
$NaH_2PO_4 \cdot 2H_2O$	0.208
$MgSO_4 \cdot 7H_2O$	0.369
Ferric citrate	0.0245
$MnSO_4$	0.002230
$CuSO_4 \cdot 5H_2O$	0.000240
$ZnSO_4 \cdot 7H_2O$	0.000296
H_3Bo_3	0.001860
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.000035
$CoSO_4 \cdot 7H_2O$	0.000028
NaCl	0.005850

*Devlin, R.M. 1967. Plant Physiology. Reinhold Co. New York.

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