GROWTH INHIBITION IN ONION (Allium cepa, L.) BY RADIATION FROM MONAZITE SAND





A

THESIS

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CERTIFICATE

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Sri P.K. Vijayan under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

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INTRODUCTION



INTRODUCTION

The importance of ionizing radiations as agents causing inhibition of mitotic activity was clearly demonstrated (Barron, 1924). These radiations cause structural changes due to breakages in the chromosomes as clearly demonstrated in <u>Drosophila melanogastor</u> (Muller, 1927, 1928; Muller and Altenberg, 1928). Gene mutations were detected on Drosophila (Muller, 1927) and on barley and maize (Stadler, 1928). These findings initiated further work on general and specific effects of radiation.

Stoppage of the synthesis of D.N.A. (Desoxyribose nucleic acid) which is important in the transference of genetic material from one cell to the daughter cells is caused. This in turn brings about the stoppage of all division and inhibition of growth. When intensity of the radiation is high, the cell division will be stopped and death will result.

Chromosome breakages are caused by radiations as a result of which cells in division may be eliminated. Union of broken chromosomes in several ways may produce structural changes. Chromosomes with such changed structures may be reproduced in mitosis and meiosis. Thus, their continuance in future generations of cells is assured. Mutation rate is accelerated as an effect of radiations. Thus, when lethal mutations are accelerated, genetical death is increased. Change in phenotype is caused and may persist in subsequent generations. Many workers have worked with radiations for inducing mutations (Kumar <u>et al.</u>, 1939).

Radiations are of two kinds, ionizing and non-ionizing. Ionizing radiations of prime interest in biological research are beta and gamma radiations of radioactive substances, X-rays and neutrons. Ultra-violet light produced by a mercury vapour lamp is the only known effective non-ionizing radiation.

The monazite sand occurring in specified areas of the sea coast in a narrow belt on the West-coast of India forms a source of natural radiation. It emits largely gamma rays and a smaller dose of beta particles. This dose is found to be 3 to 50 times the radiation naturally occurring in other areas where there is no monazite sand (Bharatwal and Vaze, 1958). Thus, the vegetation of the area is exposed to a much higher radiation than that in similar non-monazite areas. Hence, there is some possibility of the vegetation of the tract showing the effect of chronic irradiation.

Investigations were initiated by the Atomic Energy Establishment, Trombay in 1956 in order to obtain a preliminary idea of the radiation levels of the monazite areas. In 1957,

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they conducted a second survey in order to gather detailed information on the radiation levels inside living quarters. It was thus revealed that Kadiapatanam and Manavalakurichi are the two villages with the highest radiation in the tract. Investigation in the area was conducted by Nair <u>et al</u>., in 1959 to search for any possible abnormalities on plants in Manavalakurichi. Effect, if any, on the bones of rats occurring in the area was investigated by Gruneberg in 1962. A recent field study was organised by the Atomic Energy Establishment, Trombay in 1962. They conducted studies on the cytological and morphological abnormalities of low growing plants with a view to finding out their correlation with radiation dose.

The present investigation consisted of a preliminary survey of all the villages of high radio-activity in order to find out any abnormalities on plants. Studies on the effects of radiation from monazite sand on onions (<u>Allium cepa</u>, L.) were conducted by growing the crop in the close vicinity of the sand which served as the source. The resulting abnormalities on chromosomes and effect on growth of vegetative part of the plant were studied.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

A review of the extensive literature available on the mutagenic effects of radiations is not attempted. The works reported on the specific effects of radiation undertaken in the present investigation were reviewed and presented.

From the survey conducted at several places in the monazite area of Manavalakurichi it was reported that the weeds occurring in these areas were of reduced size (Nair <u>et al.</u>, 1960). These authors found that the weeds had fewer, smaller and thicker leaves, fewer branches and more stunted growth than plants belonging to the same species in non-monazite areas.

But in the experiments on the perennial grass <u>Andro-</u> <u>pogon filifolius</u> contradictory results were obtained (Mewissen <u>et al.</u>, 1959). The seeds of the grass growing in uraniferous and non-uraniferous soils from Belgian Congo were collected and grown in both uraniferous and non-uraniferous soils by the authors. They observed that the former had a higher germination percentage than those collected from and grown in non-uraniferous soils. Seedlings of the former had longer roots and greater vigour. The plants also were found to have longer stems and more vitality than the latter. These authors inferred, "the enhancement of biological potentialities than degeneration had apparently resulted from long continued low-level irradiation".

Effect on mitotic activity of the cell

It was stated that the time required by an organism to attain the adult stage is to a great extent determined by its size at maturity (Swanson, 1963). According to this, differences in size of organisms with the same cell size are due to the differences in the length of time during which cell multiplication takes place. The general relationship between mitosis and adult size is evident from this opinion.

Mitotic inhibition as an effect of ionizing radiations was clearly demonstrated (Barron, 1946; De Robertis, . 1963). The latter mentioned about this finding in his review of previous work on the effect of radiation on mitotic cell. The former reached this conclusion from his studies conducted on slices of spleen and liver of rats subjected to irradiation. According to him one of the biological effects of ionizing radiations is that they stop cellular division and result in ultimate death if vital tissues are affected in large amounts.

The most immediately observable effect of radiation on the genetic material is its inhibition of mitosis (Muller, 1954). From the studies on the effects of gamma radiation on bulbs and root crops in storage it was found that an irradiation dosage of $10 - 20 \times 10^3$ rads inhibited sprout formation in them (Salunkhe, 1961). In other experiments, gladiolus cormels having a diameter of 5 - 7 mm. were planted in rows at

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a distance of 2 to 30 meters from cobalt source and allowed to grow over a period of 4 months (Isaev <u>et al.</u>, 1960). The cormels nearest to the source received a total dosage of 28,301 r and the control 4.6 r. Sprouting was not affected by irradiation in the dosages received, but the number of leaves, height of plants and the number of newly formed cormels were found to be reduced. It was reported that when bean (<u>Phaseolus</u> <u>vulgaris</u>) seeds were exposed at 10,000 r, the plants showed deformities and slow growth (Cardona <u>et al.</u>, 1960).

Working with X-rays, it was revealed that when seedlings of <u>Vicia</u>, sunflower and mustard were irradiated death of root was evidenced (Glocker and Reuss, 1933 a, 1933 b). Death of roots was induced in <u>Vicia</u> when irradiated with X-rays, alpha rays and gamma rays (Gray and Read, 1942). The same authors in 1950 obtained by means of alpha and gamma radiations inhibition of mitosis in root cells of <u>Vicia</u>. Reduction in root growth was effected in seedlings of <u>Vicia</u> irradiated with X-ray and gamma ray both in the presence and absence of oxygen (Thoday and Read, 1949). The reduction was more in the latter case. A similar effect with X-ray was observed in <u>Allium</u> root tip.(Mallet and Perrot, 1951).

With X-rays, there is found to be a compensatory wave of cell division following depression, which indicates a stoppage of mitosis at a critical stage (Swanson, 1963). The critical stage at which mitosis is stopped was identified as the late prophase, i.e. just prior to the break down of nuclear membrane by the same author. These delayed cells then pass through the different stages of division as a group to give apparant increase in mitotic rate. The crest in mitotic frequency is, in turn, followed by a trough of it, since many of the cells that otherwise would have been in division have then, only recently entered interphase because of the previous delay (Muller, 1954). The tendency to synchronization of mitosis is thereafter expressed in a series of gradually subsiding waves of mitotic frequency.

1 Due to the above mitotic waves an accurate determination of the magnitude of the increase in cell division following irradiation is quite complicated when tissues of root tips are studied (Swanson, 1963). This is attributed to the tendency in the appearance of a reduction in mitotic rate and recovery of cells may take varying periods of time depending on the dosage employed and the sensitivity of the cells studied. With larger doses of radiation applied, the number of mitoses which are increased later in compensation will not exceed those of the normal cells as reported in several materials. With still larger doses, the return to normal may be greatly prolonged (Alberti and Politzer, 1924; Pekarek, 1927; Canti and Spear, 1929; Kemp and Juul, 1930; Spear, 1931, 1935; Transley et al., 1937; Lasnitski, 1946, 1948; Simmon Reuss et al., 1947; Knowlton et al., 1948; Knowlton and Hempleman, 1949; Carlson et al., 1949).

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Contradictory to the above, increase in the metaphases and anaphases of <u>Vicia</u> root tips after a period of 4 hours from treatment with 45 r of X-rays, at 24° C was obtained (Darlington and La Cour, 1945). Since the numbers of cells in these stages exhibited decreases at higher doses viz. 90 and 135 r at the same temperature they concluded that there is an optimum temperature and dose at which mitosis may be stimulated temporarily by X-rays. Yet in the case of <u>Vicia faba</u> root tips there is cessation of growth due to Gamma rays/X-rays/neutrons/ alpha rays at a ratio of energy dessipation equal to 9/7/1/1 (Gray and Read, 1943). In the same material mitosis remained at zero for 16, 18 and 24 hours after doses of 175 r, 420 r and 550 r respectively of X-rays were applied (Langendorff, 1930).

Radiation effect on the cell in relation to the stage of the mitotic cycle

The term mitotic cycle means the series of events which occur from the inception of one mitosis to the inception of the ensuing one. The cycle may be divided into 4 segments.

Active mitosis: That part of the cycle during which active division of the nucleus into daughter nuclei occurs.

Synthetic stage: The period following active mitosis during which major growth of daughter cell occurs. This stage involves considerable synthetic activity since the cells have to increase to double its size.

Resting phase: This stage is somewhat hypothetical stage between the end of synthesis and the beginning of high mitotic competance. This period would be when the cell most likely undergoes differentiation.

Antephase: This stage is just prior to the inception of mitosis. The characteristic increase in the volume of nucleus takes place in this stage.

The nature of changes produced by radiation depends on the stage of activity of the cell. From the point of view of mitotic inhibition meristematic tissues are highly radiosensi-tive. In general, the more active a tissue is mitotically and metabolically, the more it is radiosensitive (Swanson, 1963). In connection with inhibition of mitosis it was stated that ionizing radiations produce the effect in the following stages of the cell cycle (Muller, 1954). If a cell is already as far along in mitosis as a late prophase, metaphase, anaphase or telophase stage when radiation is applied, it will complete its division without interruption. If approaching prophase, it will be inhibited from entering this stage for a period of time that may be considerable, depending upon the material and dose. If in an early or middle prophase it may even appear to regress in phase and will then remain mitotically static until finally cells that had been in interphase during treatment have caught up with it (Muller, 1954).

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There is some difference in time found in different organisms in the period required to complete the 4 stages of division and for the daughter cell to commence the next cycle (Koller, 1947; Knowlton and Widner, 1950). Those types of cells requiring a short period to complete the cycle are more sensitive to radiation and greater damage than those with longer cycle. Radiation of dose 2500 r or more is required to kill chick tissue culture cells while they are in resting stages, but irradiation of these cells with 100 r is sufficient when they divide (Lasinitzki, 1943).

"Localised" effects of radiation occur most frequently after irradiation in cells at a stage of minimum mitotic activity and appear at post irradiation metaphase and anaphase (Carlson, 1954). Unlike the stoppage of DNA synthesis chromosome aberrations such as breaks and inversions are localised effects. But localised effects are not restricted to post-inhibition period as evidenced by the recording of acentric fragments at intervals of time shorter than those required to be produced after inhibition due to irradiation in different material. Thus, fragments appear 7½ hours after treatment in <u>Scilla</u> root tips (Marquardt, 1938), shortly after irradiation in <u>Tradescantia</u> microspores (Sax and Swanson, 1941), at 4 hours in <u>Trillium</u>, <u>Allium</u> and <u>Vicia</u> root tips (Darlington and La Cour, 1945) and at 1 hour in root tips of <u>Vicia</u> (Deufel, 1951). A dose of 250 r produces mitotic stoppage in prophase cells that are nearing the critical period before the breakage of nuclear membrane.. This is followed by reversion or simulated reversion to a stage where chromatin is similar to interphase (Carlson, 1940, 1941). Prophases are entirely absent in tissues until recovery has occurred.

Radiation effect on different stages of mitosis

In the discussion on the different stages of mitosis it was stated that other cellular functions than spindle formation are impaired by radiations (Swanson, 1963). The author also adds that the cells which have reached the critical stage of late prophase prior to the break down of the nuclear membrane are not greatly affected by radiation except as induced stickiness of chromosomes impedes chromatid separation. Those that have not reached this critical stage show a reversal of mitotic behaviour in that they regress to an earlier prophase stage. If the dosage employed is low, cells entering mitosis are not prevented from doing so, and these, together with repressed cells, then go through mitosis as a group to account for the compensatory rise in mitotic count (Swanson, 1963).

Nature of specific effects produced by radiation

Of all the metabolic reactions so far studied, the synthesis of nucleic acid seems to be the most sensitive to the

action of ionizing radiations (Barron, 1954). This inhibition was discovered by other workers (Heavy, 1945) and confirmed by others (Holmes, 1947, 1949).

This is indicated by the fact that relatively low doses of X-ray (140 r) inhibit the synthesis of DNA in <u>Vicia</u> root tip and bring about mitotic delay (Howard and Pelc, 1953). Since only cells entering mitosis synthesise new DNA (Desoxyribose nucleic acid), any interference with the mechanism involved would bring about delay. The synthetic period appears to be late interphase and the delay occurs during the period just prior to synthesis. The delay is hence brought about by failure of cells to enter the synthetic period than by the interruption of synthetic process already going on (Howard and Pelc, 1953).

Another effect on cells in mitosis is the appearance of clumped chromosomes at metaphase and an irregular separation of chromatids at anaphase. When the chromosomes appear to be pycnotic or sticky, it is termed the physiologic or primary effect of radiation (Marquardt, 1938). At metaphase, the sharp boundaries between chromosomes are lost and agglutination takes place. The effect does not prevent anaphase movement.

In a review of previous work it was mentioned that chromosome aberrations produced in chromosomes that are effectively single so far as their response to radiation is concerned

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terminal deletions (Muller, 1954). An acentric fragment, appearing double at metaphase, accompanies each of the aberrations (Swanson, 1963). In tissues such as meristematic root tips, these aberrations would tend to be eliminated and succeeding cell divisions would have fewer aberrations persisting. The formation of half or sub-chromatid breaks is doubtful. It was assumed that only chromatid breaks are possible (Ostergen and Wakonig, 1954). It was also stated that breakage by radiations in the form of chromatid and chromosome breaks must involve more than 1 or 2 longitudinal segments of the chromosome (Swanson, 1963).

The distribution of breaks involved in aberrations in Tradescantia microspore chromosomes is not at random, there being a greater frequency in the proximal than in the distal regions (Sax and Mather, 1939; Sax, 1940). The reverse of the above seems to be true for pollen tube chromosomes. In the latter, there is greater frequency in the distal ends of chromosomes than in the proximal regions. It is difficult to directly determine the initial break distribution in any organism but there is no compelling reason to believe that the initial effects of X-ray are non-random. The mechanism involved in the nonrandomness of the reunion process is not entirely clear. It was suggested that the mechanism may be stress imposed by the coiling of the chromosome, initiated possibly in the centromeric regions (Sax, 1940).

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The comparative resistance of metaphase chromosomes to breakage is, however, deceptive, for although aberrations cannot be detected initially, they appear at the next division and it now is evident that prophase and metaphase are the stages most susceptible to ionizing radiations (Swanson and Schwartz, 1953).

The yield of interstitial deletions increases as 1.5 power of the dose (Rick, 1940). When neutrons and alpha particles are used, all aberrations show a linear relation to dose (Giles, 1940,1943; Kotval and Gray, 1947). This holds true for neutrons of varying energies (Conger and Giles, 1950) as well as those released from nuclear detonations (Conger, 1954; Kirby-Smith and Swanson, 1954).

Breakage at metaphase is practically absent when observed immediately after radiation and a peak is reached at about 12 hours, corresponding to exposures made at mid or late prophase after which the frequency is achieved. The chromosomal aberrations appeared 48 hours after radiation and a steady rate of aberrations was obtained thereafter with Tradescantia (Swanson and Schwartz, 1953).

Suitability of material for study

For the study of structural changes induced by radiation, the microspore chromosomes of Tradescantia, the root tip cells of <u>Vicia faba</u> and <u>Allium cepa</u> are by far the most suitable

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materials for study (Swanson, 1963). Of the four types of cells that can be conveniently examined, the order of sensitivity from high to low is microsporocytes, microspores, root tip cells and generative nucleus in mature pollen grains (Sax and Swanson, 1941). Microspores and root tip cells of <u>Allium</u> were less sensitive than those of <u>Tradescantia</u>.

Effect of environmental conditions on radiation sensitivity

Muller (1954) summarising previous work has mentioned that the production of chromosome breaks by the presence of oxygen during exposure to ionizing radiations is well established. Oxygen must be present during radiation to be effective; pre or post changes in oxygen tension on irradiated cells were without effect. This is also concerning rates of mitosis, stickiness of chromosomes, lethality and nucleic acid polymerisation. It is seen that anoxia is a very effective means of reducing radiation damage (Swanson and Johnston, 1955). Anoxia has much less an effect when alpha rays rather than X-rays are employed (Thoday and Read, 1949), while neutrons show an intermediate relationship (Giles et al., 1952). The ion density of the radiation will, therefore, determine the magnitude of the oxygen effect. On the whole, the most suitable hypothesis is that oxygen tension in determining the frequency of aberrations, does so by modifying the rates of breakage (Gibs, 1954; Swanson, 1955; Gray, 1953; Korah, 1959). But the studies made by Wolff (1954) make it clear that both breakage and rejoining are oxygen dependent.

MATERIALS AND METHODS

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MATERIALS AND METHODS

Materials:

In the present experiment, the effect of radiation on growth inhibition was studied on onion (<u>Allium cepa</u>, L.). Monazite sand which gives out gamma rays and beta particles was used as the source of radiation.

Onion was selected as the material, for study for the following special advantages:

It has been employed by several workers previously for radiation studies. The roots grow in glass tubes filled with water rapidly without the formation of secondary roots for 10 to 12 days during wich period they could be directly irradiated. Measurements and other observations could be taken removing the bulbs and if necessary spreading the roots. Root tips form convenient material for cytological examination. The crop in the field grows without much lateral spread so that a vertical column of sand can irradiate the growing point at all stages. The crop flowers early and matures rapidly so that final yield is obtained within a short period.

Vegetative propagation system of the crop ensures uniformity of material obtained for the study. Since onion is not a cultivated crop in the monazite beach, original market sample used will be free from effects of monazite radiations. Monazite sand with a sufficiently high degree of purity with respect to uranium and thorium was brought from the Travancore Monazite Factory in Chavara and the Rare Earth Factory in Manavalakurichi. Since phosphate in the sand formed a nutrient, direct contact with the roots was avoided in the experiment. In order to measure and adjust the deflection caused by the source to 49 microamperes, as compared to 5 microamperes of background radiation, Geiger counter TR 60 type of the Atomic Energy Commission, Trombay was utilised.

Methods:

The investigations were carried out in four stages:

i. Preliminary survey of the monazite area was conducted in order to assess the nature and kind of crops growing. Low growing plants were observed for stunted growth.

ii. Laboratory studies to assess the effect of radiation on the number of roots and also on root elongation.

iii. Field study to find out the effect of radiation on vegetative growth and yield.

iv. Cytological observations 'invitro' to spot out chromosome aberrations, if any, in the dividing cells of onions.

In the preliminary survey conducted in the monazite areas, the coastal villages covered are the following:-

- 1. Manavalakurichi
- 2. Kadiapatanam
- 3. Muttam
- 4. Kovalam
- 5. Chavara 🗤

These places were reported to be radioactive by the Atomic Energy Establishment, Trombay in 1958. Since <u>Phaseolus</u> <u>trilobus</u> occurs largely in the monazite area, representative samples of this plant were collected. The range in length of peduncle and pethole was worked out in inches. The measurement was compared with the description available for the normal measurements of the plant.

In the laboratory and field trials, the experiment was conducted adopting a split-plot design.

Main plots -	2; radiation <u>versus</u> no radiation.
Sub-plots -	6; weight groups of onions of
	2 gm., 3 gm. upto 7 gm.
Replication -	9; each initiated on different
	dates.

In the laboratory studies, six glass specimen tubes of 2.5 cm. diameter and 10 cm. height filled with water, were arranged in a circular manner. A taller tube filled with monazite sand was placed in the centre. By this arrangement all the water tubes are at equal distance from the central tube containing the source of radiation. Onion bulbs were weighed and then grouped into 6 weight groups of 2 gm., 3 gm., 4 gm., upto 7 gm., correct to 0.1 gm. The onions of the different weight groups were allotted to the water tubes at random. The bulbs were arranged over the water tubes in such a way that their bottom levels were kept in contact with water. The central tube which contained monazite sand provided the main source of radiation. The source was so adjusted that a uniform deflection of 49 microamperes, as measured by the Geiger counter TR 60, was obtained for each of the onions. Since the container of the source was kept vertically, the growing tips of roots and shoots were always at uniform distance from it. The non-irradiation control was similarly arranged and ordinary sand was kept in the central tube (Fig. 1) instead of the monazite sand. Nine replications were set and each was started on a different date. The layout of the experiment with nine replications is diagrammatically represented in Fig. 2. The date of commencement of root formation as indicated by the appearance of a white speck at the bottom of the bulb was recorded. The photographs of non-irradiated and irradiated bulbs after the initiation of root growth are presented in Fig. 3 A and 3 B respectively.

On the 8th day after setting the experiment, the roots on the bulbs were spread out on moist glass sheet for measurement. The length of each root was measured in millimeters. The

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mean length per root per bulb was computed, tabulated and statistically analysed. The number of roots per bulb was tabulated and analysed.

Field studies:

The same layout employed for laboratory studies was adopted for the field studies. The latter is represented diagrammatically in Fig. 4. The onions after root measurement on the 8th day in the laboratory were planted in pots in the field. The 9 replications of the non-irradiated and irradiated plants standing in the field in pots are presented in Fig. 5 A and 5 B respectively. Sand was provided in glass jars in the centre of each circle in the pots. This was done to avoid mixing up of the sand with the soil in the pot and avoid any chemical action causing growth inducement. The source was so adjusted to show a deflection of the same amplitude as in the laboratory for each plant.

The height of individual plants in all treatments was measured in millimeters from 2nd upto 8th week at weekly intervals. The photographs of the non-irradiated and irradiated plants in pots are presented in Fig. 6 A and 6 B respectively. The number of leaves on the corresponding days was counted. The data in respect of these two observations were tabulated and statistically analysed. At maturity, the bulbs of individual treatments were collected separately. The bulbs were cleaned, air dried and final yield recorded correct to the first decimal place. The results were tabulated and statistically analysed.

Flowering dates were noted for individual plants.

In the cytological studies, a glass tube of 9 mm. diameter and 6.5 cm. length was filled with water and immersed in monazite sand and onion kept over it for germination. Thus, radiation was provided all around the elongating roots. Similar method with ordinary sand was adopted for the control. On the 5th day of setting, roots from both the bulbs were cut and fixed in Carnoy's fluid (6:3:1) for 2 hours. The roots were hydrolysed in normal hydrochloric acid at 60°C for 5 minutes and root tips stained with acetocarmine. Squashes were prepared and anaphase chromosomes examined for chromosomal aberrations.

RESULTS

RESULTS

The results of the survey and the investigations conducted on the effects of monazite sand on onions are presented below.

I. Survey:

The survey conducted in the monazite area revealed that among the many plants growing in the tract, <u>Phaseolus</u> <u>trilobus</u> occurs in large numbers. This plant was found to have stunted growth. Measurements of petiole and peduncle length in plants selected at random were recorded. The data were tabulated and are presented below in comparison with the standard measurements of the species.

Part of the plant	Range in inches	
	Normal	From monazite
Petiole length	l to 1.50	0.25 to 1.06
Peduncle length	4 to 13	0.50 to 1.56

The range in length of petiole and peduncle was lower for the plants collected from the monazite area than those from normal areas. In the case of peduncle-length the reduction is more conspicuous. This gives an indication of reduced size of the plants growing in the monazite sand area.

II. Laboratory studies:

i. Time required for root formations:

The record of the number of days required for root formation (Table I) reveals no difference between the non-irradiated and the irradiated bulbs. It is concluded that irradiation does not inhibit initial root formation.

ii. Number of roots:

The observations for the number of roots obtained from the same experiment were statistically analysed and the analysis of variance presented in table II..

It is found that the main plot effect is significant at 5 per cent level while the sub-plots and interaction effects are significant at 1 per cent level.

(a) Comparison of main plot treatments (Irradiation \underline{vs} . no irradiation).

The mean number of roots per bulb for the main plot are given below:-

Treatment	Mean number of roots per bulb.	Standard error for the compari- son of the treat- ment means.	Critical difference
No radiation Irradiated	41.7 35.8	1.52	3,5

Thus the mean number of root per bulb is significantly low in irradiated treatment than in the non-irradiated one. Hence the radiation is found to be effective in reducing the number of roots produced.

(b) Comparison of sub-plot effects (weight groups).

The mean number of roots per bulb for the different weight groups are given below:-

Weight groups in gm.	Mean number of roots per bulb	Standard error for comparison	Critical dif- ference at 5 per cent level
7	51.1		
5	47.4		
6	41.8		
4	35.1	4.44	8.70
З	34.9		
2	22.3		

Low numbers of roots are produced by the 2 gm. group while 3 gm., 4 gm., and 6 gm. are on par. The weight groups 7 and 5 are on par.

iii. Length of roots:

The analysis of variance for the mean length per root per bulb on the eighth day of irradiation is presented as table III.

It is found that the main plot and sub plot effects are significant while interaction effects are not significant.

(a) Comparison of main plot treatments (irradiation <u>vs</u>. no irradiation):

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Treatment	Mean length	Standard	Critical dif-
	of roots per	error for	ference at 5
	bulb in mm.	comparison	per cent level
No irradiati on Irradiation	54.5 48.0	1.64	3.78

The comparison is made with the main plots averaged over six weight groups. The treatment means are significantly different and it can be seen that the mean length per root per bulb of irradiated plots is lower. This suggests that radiation is effective in inhibiting the growth of roots. (b) Comparison of sub-plot treatments (weight groups):

The mean length per root per bulb in the different weight groups are presented below:-

Weight groups (gm.)	Mean length of root per bulb in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
7	63.8		
6	58 .5		
5	54.8		
4	49.0	3.35	6 .57
З	44 .2		
2	37.2		

Conclusion: 7 6 5 4 3 2

The weight groups 7 gm. and 6 gm. produce the maximum mean length per root per bulb whereas the weight group 2 gm. produces the minimum length. The comparison reveals that the mean length per root produced by a bulb is dependent on the initial weight of the bulb and a bulb with a higher weight produces larger mean length per root than a bulb with lower weight.

III. Field studies:

(a) <u>Height of plants</u>:

The plant height measurements from the second week to eighth week at weekly intervals after planting in the field were analysed and the tables of analysis of variance presented (Tables IV to X).

From the tables it is seen that main (irradiation <u>versus</u> no irradiation) effects are not significant at any of the growth stages while the sub-plot (weight group) effects are significant in all observations. In the case of interaction, the effect is significant at only one stage of observation, viz., at six weeks after planting.

i. Comparison of weight groups in the second week of planting:

The height of plants in different weight groups are furnished below:-

27.3

The groups 4 gm., 2 gm., 3 gm., and 5 gm. are on par and 5 gm., 7 gm. and 6 gm. are on par.

ii. Comparison of weight groups in the third week of planting:

The mean height of plants in different weight groups are furnished below:-

Weight groups in gm.	Mean height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
6	222.4		
7	220.1		
5	206.8	11.75	30.3
4 [,]	187.1		
3	185.7		
2	176.6		
••••••••••••••••••••••••••••••••••••••	Conclusion:	6754	3 2

The weight groups 2 gm., 3 gm., 4 gm. and 5 gm. are on par. The weight groups 6, 7 and 5 have given the highest height of plants. iii. Comparison of weight groups in the fourth week of planting:

The mean height of plants in the different weight groups are furnished below:-

Weight groups in gm.	Height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
6	260.5		
7	253.3		
5	243.7	9.03	23.82
3	231.2		
4	224.9		
2	219.1		
	Conclusion:	<u> </u>	4 2

It is seen from the table that the weight groups 2 gm., 4 gm. and 3 gm. are on par, 5 gm. and 6 gm. are on par while 3 gm., 5 gm. and 7 gm. are found to be statistically equal.

iv. Comparison of weight groups in the fifth week of planting:

groups:

Weight groups in gm.	Mean height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
7	299.5		
6	299.4		
5	283,4		
3	267.1	11.20	29.55
4	262.0		
2	255.9		

Conclusion: 7 6 5 3 4 2

It is seen that the weight groups 2 gm., 4 gm., 3 gm. and 5 gm. are on par and significantly different from 6 gm. and 7 gm.

v. Comparison of weight groups in the sixth week:

Mean height of plants in the different weight groups:

Weight groups in gm.	Mean height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
7	320.6		
6	316.5		
5	294.4		
3	281.3	10.27	27.09
2	281.6		
4	280.3		

Conclusion: 7 6 5 3 2 4

The weight groups 4 gm., 2 gm., 3 gm. and 5 gm. are on par and 6 gm. and 7 gm. are on par.

vi. Comparison of height of plants in the different weight groups at seven weeks after planting:

Weight , groups in gm.	Mean height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
6	328.1		
7	323.1		
5	313.7	12.79	25.07
2	295.2		
4	291.7		
З	290.3		

It is seen that the weight group 3 gm., 4 gm. and 2 gm. are on par and 5 gm., 7 gm. and 6 gm. are on par.

vii. Comparison of weight groups at eight weeks after planting:

The mean height of plants in different weight groups at eight weeks after planting:

Weight groups in gm.	Mean height of plants in mm.	Standard error for comparison	Critical dif- ference at 5 per cent level
7	329.8		
6	329.0		
5	317.2	11.58	22.7
2	302.1		
3	301.1		
4	300.5		

Conclusion: 7 6 5 2 3 4

It is found that the weight groups 4 gm., 3 gm., 2 gm. and 5 gm. are on par with each other and significantly lower than 6 gm. and 7 gm. which are also on par. viii. Comparison of height of plants in all the seven observations:

The mean height of plants corresponding to irradiated and non-irradiated treatments in all the observations are tabulated and presented below. The general mean for the growth per week as well as the predicted mean are given in the table below:-

Weeks after planting	Mean height Non-irradiated		General mean	Predicted heights
2	168.6	16 1.6	165.1	160.8
3	201.7	197.9	199.8	205.4
4	241.7	235.9	238.8	242.4
5	281.6	272.5	277.1	271.8
6	298.1	291.7	294.9	293.6
7	308.6	305.4	307.0	307.8
8	321.3	305.4	313.4	314.4

It may be seen from the table that the mean height of plants in the non-irradiated plots in all the 7 observations is always more than that of plants in the irradiated plants. But the difference at no stage of growth is significant. The mean height of plants averaged over the six weight groups of onions for the non-irradiated and irradiated are represented in bar diagram in Fig. 7. The growth lines for the seven weeks are presented in Fig. 8.

(b) <u>Number of leaves</u>:

The data on the number of leaves in each plant recorded in observations from second to eighth week at weekly intervals were analysed and the tables of analysis of variance presented as tables XI to XVII. The analyses reveal that the main plot (irradiation \underline{vs} . non-irradiation) effects are not significant at any of the observations. The sub-plot (weight groups) effects are significant at all observations. Interaction effects are not significant in all the observations except at the three week stage of growth.

(i) Comparison of sub plot (weight group) effects at two weeks after planting:

The average number of leaves in the different weight groups is presented below:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
6	20.3		\ \
7	20.2		
5	17.2	١	
3	12.9	1.7	3.38
4	12.6		
2	10.7		

Conclusion: 6 7 5 3 4 2

It is found that the weight groups 2 gm., 4 gm. and 3 gm. are on par and significantly lower than 5 gm., 7 gm. and 6 gm. which are on par.

(ii) Comparison of weight groups at third week after planting:

The mean number of leaves in the different weight groups are given below:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
7	27.8		
6	26.3		
5	24.0	1.52	4.01
3	19.0		
4	18.0		
2	15.1		
		·····	
	Conclusion:	76534	2

The treatments 7 gm. and 6 gm. are on par and significantly higher than 3 gm., 4 gm. and 2 gm. which are on par. The weight groups 6 gm. and 5 gm. are on par. (iii) Comparison of weight groups on the fourth week after planting:

The mean number of leaves in the different weight groups are presented below:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
7	34.9		
6	33.7		
5	32.1	2.29	5.90
З	24.6		
4	2 2.8		
2	18.8		

Conclusion: 7 6 5 3 4 2

It is seen from the above that the weight groups 2 gm., 4 gm. and 3 gm. are statistically on par and significantly lower than 5 gm., 6 gm. and 7 gm. which are on par.

(iv) Comparison of weight groups on the fifth week after planting:

The mean number of leaves in the weight groups are

presented below:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Criticial dif- ference at 5 per cent level
7	41.0		
6	39.7		
5	38.5	2.94	5.76
3	28.7		
4	28,4		
2	23.3		

Conclusion: 7 6 5 3 4 2

It is found from the above that the weight groups 2 gm., 4 gm. and 3 gm. group are on par and lower than 5 gm., 6 gm. and 7 gm. groups which are on par.

(v) Comparison of weight groups on the sixth week after planting:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
7	46.7		
6	42.3		
5	42.1	3.37	8,68
4	33.3		
3	32.1		
2	25.6		

The weight groups 2 gm., 3 gm. and 4 gm. are found to be statistically equal and significantly lower than 5 gm., 6 gm. and 7 gm. which are on par with each other.

(vi) Comparison of weight groups at seventh week after planting:

The mean number of leaves on the different weight groups are presented below:

Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
48.1		
45.1		
45.1	3.69	7.23
35.8		
32.1		
28.8		
Conclusion	7 6 5 3	4 2
	of leaves per plant 48.1 45.1 45.1 35.8 32.1 28.8	of leaves error for per plant comparison 48.1 45.1 45.1 3.69 35.8 32.1 28.8

Weight groups 2 gm., 4 gm. and 3 gm. are statistically equal while 5 gm., 6 gm. and 7 gm. are also on par. (vii) Comparison of weight groups on the eighth week after planting:

The mean number of leaves obtained for the weight `groups are presented below:

Weight group in gm.	Mean number of leaves per plant	Standard error for comparison	Critical dif- ference at 5 per cent level
7	51.4		
5	4 6 .8		
6	42.9	3.79	9.76
3	38.4		
4	33.2		
2	30.1		

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Conclusion: 7 5 6 3 4 2

It is seen that the treatment groups 2 gm., 3 gm., 4 gm. are on par and significantly lower than 6 gm., 5 gm. and 7 gm. which are on par.

Summary table for number of leaves:

Summary table for the mean number of leaves are presented below:

Week after planting	Non-irradiated	Irradiated
2	16.2	15.1
3	22.0	21.7
4	27.9	27.8
5	33.3	33.1
6	37.2	36.1
7	39.5	38.5
8	43.1	37.8

Number of leaves are on the increase as the weeks proceed but rate of increase is reduced during last stages of growth. The mean number of leaves per plant averaged over the six weight groups is represented as bar diagram in Fig. 9.

(c) <u>Yield of bulb</u>:

The yield data were statistically analysed and table of analysis of variance is presented (Table XVIII).

It is seen that the main (irradiation \underline{vs} . no irradiation) effect is significantly high while the weight groups and interaction are not significant. (i) Comparison of main plot:

(Irradiation vs. no irradiation)

Mean yields of onions in grams from irradiated and non-irradiated groups are given below:

Treatment	Mean yield of bulbs in gm.	Standard error for comparison of means	Critical dif- ference at 5 per cent
Non-irradiated	51.7	0.00	0.01
Irradiated	38.6	3.82	8.81

The difference between the means is found to be much higher than the critical difference. The mean weight of bulbs in the irradiated plots is therefore significantly lower than in non-irradiated plots.

IV. Cytological observations:

Breakage of chromosomes:

Cells in metaphase found in squash preparations of root tips from irradiated bulbs exhibited clumping of chromosomes. Breakage was exhibited in anaphase chromosomes. Two adjoining cells with broken chromosomes are presented in Fig. 10. Cells in mitosis from root tips collected from non-irradiated bulbs are presented in Fig. 11 for comparison. Since several such breakages are seen in one cell and no breakages in control, irradiation is found to be effective in inducing chromosome breakages.

V. Ancillary observations

(i) Club shaped roots:

In the laboratory studies club shaped appearance of the ends of roots were seen in certain of the irradiated bulbs. Further growth was inhibited in such roots.

(ii) Chlorophyll colour:

Green colour of leaf was found to be less intense for the irradiated plant than the control in the field suggesting the effect of radiation on chlorophyll organisation. The specific effect can be ascertained by conducting elaborate experiments.

(iii) Flowering:

The number of inflorescences formed in each treatment and their dates of emergence were recorded and presented as table XIX. One out of 54 plants produced inflorescence in the control (Fig. 5 A), whereas seven out of 54 plants in the irradiated treatment produced inflorescence. A close view of the inflorescence borne on the irradiated plant is presented in Fig. 6 B. The difference in number of inflorescences between irradiated and non-irradiated treatments is presented in Fig. 6 B.

DISCUSSION OF RESULTS

DISCUSSION OF RESULTS

The highlights of the experimental results obtained during the present investigation with regard to the effects of radiation from monazite sand on the growth and cell division of <u>Allium cepa</u>, L. are discussed here.

Survey

Survey of natural vegetation was conducted in the villages of Kadiapatanam, Manavalakurichi, Muttam and Midalam. According to the survey report of the Atomic Energy Establishment, Trombay it was found that these areas are of high gamma activity amounting to an annual dose of 1,753 to 2,814 millirads. The survey in the area revealed that <u>Phaseolus trilobus</u> occurs in this area in abundance as a dryland weed. It appeared that this weed had a stunted growth and a comparison of the lengths of petiole and peduncle in random plants collected from the area with standard measurements for the species (Thandulingam, 1955) confirmed this observation. This is in agreement with the observation of Nair <u>et al</u>., (1960) that there is a general reduction in the size of weeds occurring in monazite sand area.

But Mewissen <u>et al</u>., (1959) in their studies on <u>Andropogon filifolius</u> obtained contradictory results and reported that chronic low level irradiation is capable of

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stimulating growth. The stunting effect on growth obtained in this survey can be attributed to the high continuous natural radiation present in the monazite sand area.

It is found that natural radiation from monazite sand giving a deflection of 49 microamperes in the Geiger counter is effective in reducing the number of roots produced by onion bulbs. This statement holds true irrespective of the weight of onion bulbs. Meristematic tissues are highly radiosensitive (Swanson, 1963). Irradiation inhibited sprout formation in bulbs, root crops and tubers in storage (Salunkhe, 1961). These informations make it possible to conclude that radiation even at such a low intensity is capable of affecting the growth and initial development of the root primordium which is essentially a meristematic tissue.

Root elongation

The results obtained in the present investigation reveal that the mean length of a root in a bulb is effectively reduced by irradiation. This statement holds good irrespective of the initial weight of the bulb and the number of roots produced. Root growth in onion being mostly linear and roots generally having uniform thickness, the length of root can be taken as an index of mitotic activity provided the size of the cell remains constant. Therefore, it can be reasonably assumed that the reduced length of root in irra-

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diated bulbs is a result of reduced rate of mitosis in comparison to the normal mitotic rate in roots of non-irradiated bulbs. One of the possible causes of a reduced mitotic rate is a partial inhibition of mitotic activity brought about by radiation. Such inhibitions have been reported by several investigators in various plants.

Retardation of root growth in seedlings of wheat of variety Big Club was reported to be produced by X-ray (Zirkle et al., 1937) and similar effect in wheat of Nabob variety by X-ray (Zirkle and Lampe, 1938). Death of roots was induced in <u>Vicia</u> when irradiated with X-rays, alpha rays and gamma rays (Gray and Read, 1942). Reduction in root growth was effected in seedlings of Vicia irradiated with X-ray and gamma ray both in presence and absence of oxygen (Thoday and Read, 1949). Inhibition of mitosis in root cells of Vicia was obtained by means of alpha and gamma radiation (Gray and Read, 1950). Reduction in growth of Allium root was effected (Mallet and Perrot, 1951). The report that the most immediately observable conspicuous effect of radiation is inhibition of mitosis (Muller, 1954), is also in favour of this view.

Thus, the evidence obtained from a comparative study of the number of roots and mean length of root in irradiated and non-irradiated bulbs makes possible a general statement that natural radiation even at a low intensity is capable of inhibiting the development and initial growth of root primordia and also is effective in partial inhibition of mitotic activity in root.

Effect on mitotic chromosomes

Radiation was found to be effective in inducing clumping of metaphase chromosomes. Similar observations were reported by other workers (Marquardt, 1938). This author reported that at metaphase, the sharp boundaries between chromosomes are lost and agglutination takes place. The effect did not prevent anaphase movement. Another effect reported by the same author is the appearance of clumped chromosomes at metaphase and irregular separation of chromatids at anaphase.

One of the cytological observations in the present investigation is the appearance of broken chromosomes in anaphase (Fig. 10). Chromosome abnormalities in root cells of seedlings of bean (<u>Vicia</u>) were produced by X-ray (Marshak, 1939, 1942) and in those of pea and tomato seedlings (Marshak, 1939). Chromatid breaks and chromosome breaks were produced by X-ray in <u>Tradescantia</u> flowers (Thoday, 1942). When cells are irradiated at metaphase, the breakage of chromosomes may become evident in anaphase (Swanson, 1963). According to this author, this is because, due to agglutination of chromosomes, the broken fragments are not separated to become visible immediately. Since the agglutination effect does not prevent anaphase movement, the centric fragments move towards either of the poles and the acentric ones which are not pulled towards either of the poles lag behind and are separated from the centric fragments (Swanson, 1963).

Thus, a comparative study of the different mitotic stages in the irradiated and non-irradiated root tips revealed that radiation is effective in inducing clumping of metaphase chromosomes and breakage of anaphase chromosomes.

When a cell containing chromosome fragments divides, any acentric pieces, or acentric isochromosomes lacking a spindle fibre attachment, fail to become transported to either daughter nucleus. In consequence, the descendant cells are aneuploid, lacking this portion of one of their chromosomes and for this reason are abnormal. If the missing part is large and important enough this deficiency can even cause the death of the cell. Dicentric bridge, if formed, will be pulled both ways in division resulting in the death of the cells involved (Pontecorvo and Muller, 1941). Thus, breaks in chromosomes if they occur in large numbers of cells may result in the ultimate growth inhibition as evidenced in the roots of onions in the present instance.

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Effect of radiation on the height of plants and number of <u>leaves</u>

Observations on plant height and number of leaves in onion reveal that radiation is not effective in inducing changes in these characters at any stage of growth. The summary table for mean heights (on page No. 33) reveals that the mean height of non-irradiated plants is greater than the mean heights of irradiated plants at all stages of growth. The slow growth of plants evidenced in the irradiation studies with X-ray on bean (Phaseolus vulgaris) seeds of four varieties (Cardona et al., 1960) is a similar instance. In the present experiment though the differences between means at corresponding stages of growth are not statistically significant, the figures obtained suggest a positive trend always in favour of the non-irradiated treatments. This might be due to the low intensity of radiation employed. The evidence obtained therefore makes it reasonable to infer that radiation of a higher intensity or dose may possibly be effective in reducing the height of plant.

Effect of radiation on the yield of bulbs of onions.

Yield of onions was lower in the irradiated than in the non-irradiated group in the present experiment. Prolonged irradiation with Cobalt 60 affecting the yield of gladiolus cormels (Isaev <u>et al.</u>, 1960) is a similar instance. Hence the results suggest that radiation even at a low intensity of a deflection of 49 microamperes reduces the yield.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The first part of the present investigation is a survey of the effect of radiation on natural vegetation in the monazite sand area.

Studies under controlled conditions were undertaken to ascertain the effect of natural radiation from monazite sand on growth and yield of onions and that on mitosis in root tip cells. The experiment was conducted with different weight groups of onions adopting a split plot design. The results obtained were analysed according to the analysis of variance method and conclusions drawn. The important results obtained are summarised below.

The survey revealed that <u>Phaseolus trilobus</u> occurring in the monazite area exhibited stunted growth and these plants had shorter petiole and peduncle than standard plants. This stunting may be attributed to be due to the high dose of natural radiation in the area.

Natural radiation from monazite sand even at a low intensity is capable of inhibiting the development of root primordia and also is effective in partial inhibition of mitotic activity in roots of onion.

Cytological observations of mitotic chromosomes from irradiated root tip cells of onion suggest the effective-

ness of radiation even at a low intensity to induce clumping of metaphase chromosomes and breakage of anaphase chromosomes.

The results of field studies suggest that radiation at low intensity is not effective in inhibiting the growth of shoot and in reducing the number of leaves. But the trend of the result favours the idea that radiation at a higher dose may be effective in reducing the height of plants.

Radiation was also found to be effective in reducing the yield of onions.

TABLE-I

Table showing the number of days required for initiation of root formation from the date of setting up of the experiment

Replication	Number of days for root formation		
	Non-irradiated	Irradiated	
I	2	3	
II	2	2	
III	1	З	
IV	2	2	
v	3	3	
VI	1	3	
VII	l	3	
VIII	2	1	
IX	1	2	

TABLE - II

Table of analysis of variance for the number of roots per bulb in the laboratory

Source of variation	Degrees of freedom	Sums of squares	Mean square	Variance ratio
Replication	8	337.08	42.21	
Whole plots	1	375.96	375.96	6.00 *
Error (a)	8	501.36	62.67	
Weight groups	5	4,614.95	722.99	4.06 **
Interaction	5	3,642.90	728.58	4.09 **
Error (b)	80	14,239.42	177.99	
			,	
Total	107	23,711.67	,	

* Indicates that the results are significant at 5 per cent level.

TABLE - III

Analysis of variance table for the mean length per root per bulb in the laboratory

Source of variation	Degree of freedom	Sums of squares	Mean square	F. value
Replication	8	1,450.00		
Whole plot	1	435.75	435.75	6.01 *
Error (a)	8	580.01	72.50	
Sub-plot	5	2,017.75	403.55	4.00 **
Interaction	5	1,060.00	212.00	2.10 Not si nifica
Error (b)	80	8,068.04	100.85	
Total	107	13,611.55		

* Indicates that the results are significant at 5 per cent level.

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TABLE - IV

Table of analysis of variance for the height of onions on the second week after planting (in millimeters)

Source of variation	Degrees of freedom	f Sums of squares	Mean squar e	Variance ratio
Replication	8	25,295.00	3,161.88	
Whole plot	1	1,323.00	1,323.00	0.91
Error (a)	8	11,601.34	1,450.17	
Weight groups	5	21,337.56	4,267.51	4.21 **
Interaction	5	1,988.33	397.67	0.39
Error (b)	80	81,021.44	1,012.77	
Total	107	1,42,566.67		

TABLE-V

Table of analysis of variance for the height of plants on the third week after planting (in millimeters)

Source of variation	Degree of freedom	Sums of squares	Mean square	F. value
Replication	8	54,042.19	6,755.27	
Whole plot	l	385.33	385.33	0.09
Error (a)	8	33,373.33	4,171.67	
Weight groups	5	33,649.99	6,729.10	5.42 **
Interaction	5	2,657.31	531.46	0.43
Error (b)	80	99,369.37	1,242.12	
Total	107	2,23,477.52		

TABLE - VI

<u>Table of analysis of variance for the height of onions</u> on the fourth week after planting (in millimeters)

Source of variation	Begrees of freedom	Sums of squares	Mean square	F. value
Replication	8	75,576,96	9,447.12	
Whole plot	1	907.12	907.12	0.04
Error (a)	8	2,06,596.79	25,824.60	
Weight groups	5	24,222.71	4,844.54	6.44 **
Interaction	5	4,883.16	976.63	1.29
Error (b)	80	60 ,149.0 0	751,86	
Total	107	2,43,037.21		

TABLE - VII

<u>Table of analysis of variance for the height of plants</u> <u>on the fifth week after planting (in millimeters)</u>

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	81,674.17	10,209.27	
Whole plot	1	2,196.01	2,196.01	0.53
Error (a)	8	33,374.05	4,171.76	
Weight groups	5	32,316.45	6,463,29	5.72 **
Interaction	5	2,531.15	506.23	0.44
Error (b)	80	90,341.76	1,129.27	

Total	107	2,78,790.92		

TABLE - VIII

Table of analysis of variance for the height of onions on the sixth week after planting (in millimeters)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	54,362.19	6,795.27	
Whole plot	1	1,102.10	1,102.10	0.13
Error (a)	8	68,488.64	8,561.08	
Weight groups	5	25,809.05	5,161.08	5.44 **
Interaction	5	28 ,833.6 6	5 ,7 66 .73	6.00 **
Error (b)	80	75,933.13	949.16	
Total	107	2,54,528.77		

TABLE - IX

Table of analysis of variance for the height of plants on the seventh week after planting (in millimeters)

Source of Variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	53,029.18	6,628,65	
Whole plot	1	1,381.86	1,381.86	0.17
Error (a)	8	64 , 512 .8 9	8,064.11	
Weight groups	5	25,410.82	5,082.16	3.45 **
Interaction	5	4,327.86	865.57	0.58
Error (b)	80	1,17,809.49	1,472.62	
Total	107	2,66,472.10		

TABLE - X

<u>Table of analysis of variance for the height of onions</u> on the eighth week after planting (in millimeters)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	43,624.00	5,458.00	
Whole plot	1	7,701.34	7,701.34	1.27
Error (a)	8	48,516.33	6,064.54	
Weight groups	5	15,765.89	3,153.18	2.61 *
Interaction	5	10,615.88	2,123.18	1.76
Error (b)	80	96,733.23	1,208.16	
Total	107	2,22,956.67		********

TABLE - XI

Table of analysis of variance for the number of leaves on the second week after planting

Source of variation	De gre es of freedom	Sums of squares	Mean square	F. value
Replication	8	1,968.56	246.07	
Whole plot	1	31.08	31.08	1,42
Error (a)	8	175.59	21.95	
Weight groups	5	1,444.34	288.87	11.54 **
Interaction	5	151.58	30.32	1.21
Error (b)	80	2,001.41	25.02	
Total	107	5,772.56		

TABLE - XII

Table of analysis of variance for the number of leaves on the third week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. ratio
Replication	8	2,074.91	3	
Whole plot	1	24.00	24,00	0.45
Error (a)	8	429.25	53,6 6	
Weight groups	5	3,438.38	687,68	33.25 **
Interaction	5	414.63	82.93	4.01 *
Error (b)	80	1,654.49	20,68	

Total	107	8,035.66		

- * Indicates that the results are significant at 5 per cent level.
- ** Indicates that the results are significant at 1 per cent level.

TABLE - XIII

Table of analysis of variance for the number of leaves per plant on the fourth week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	1,362.97		
Whole plot	1	1.63	1.63	0.02
Error (a)	8	702.70	89.09	
Weight groups	5	3,915.41	783,08	16.60 **
Interaction	5	333.70	66 .7 4	1.41
Error (b)	80	3,774.84	47.18	
Total	107	10,091.30		

** Indicates that the results are significant at 1 per cent level.

TABLE - XIV

Table of analysis of variance for the number of leaves on the fifth week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	907.91	113.49	
Whole plot	1	4.29	4.29	0.04
Error (a)	8	850.54	106.32	
Weight groups	5	4,899.52	979.90	12.59 **
Interaction	5	435.04	87.01	1.12
Error (b)	80	6,228.44	77,86	
Total	107	13,325.74		

** Indicates that the results are significant at 1 per cent level.

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TABLE - XV

Table of analysis of variance for the number of leaves on the sixth week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	1,216.19	152.02	
Whole plot	1	2 9. 04	2 9. 04	0.18
Error (a)	8	1,295.63	161.95	
Weight groups	5	6,017.08	1,203.42	11.76 **
Interaction	5	603.07	120.61	1.18
Error (b)	80	8 ,181.51	102.27	
Total	107	16,742.52		

** Indicates that the results are significant at 1 per cent level.

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TABLE - XVI

Table of analysis of variance for the number of leaves on the seventh week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	1,586.29	198.29	
Ma in plot	l	25.03	25.03	0.08
Error (a)	8 ·	2,338.97	292.37	
Weight groups	5	5,500.85	1,100.17	8.98 **
Interaction	5	1,066.97	213.39	1.74
Error (b)	80	9,799.85	122.50	
Total	107	20,317.96		

** Indicates that the results are significant at 1 per cent level.

TABLE - XVII

Table of analysis of variance for the number of leaves on the eighth week after planting

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	3,627,63	453.45	
Whole plot	1	524.59	524.59	2,56
Error (a)	8	1,635.74	204.47	
Weight groups	5	5,911.96	1,182.39	9.41 **
Interaction	5	850.63	170.13	1.3
Error (b)	80	10,362.41	129.53	
Total	107	22,912.96		

** Indicates that the results are significant at 1 per cent level.

TABLE - XVIII

Table of analysis of variance of weight of onions per bulb (in grams)

Source of variation	Degrees of freedom	Sums of squares	Mean square	F. value
Replication	8	4,975,72		
Whole plot	1	4,657.08	4,657.08	11.25 *
Error (a)	8	3,313.03	414.13	
Weight groups	5	8,399.67	1,679.93	80.0
Interaction	5	2,20,627.59	44,125.52	2.21
Error (b)	80	15,95,329.60	19,941.62	
Total	107	18,37,302.65		

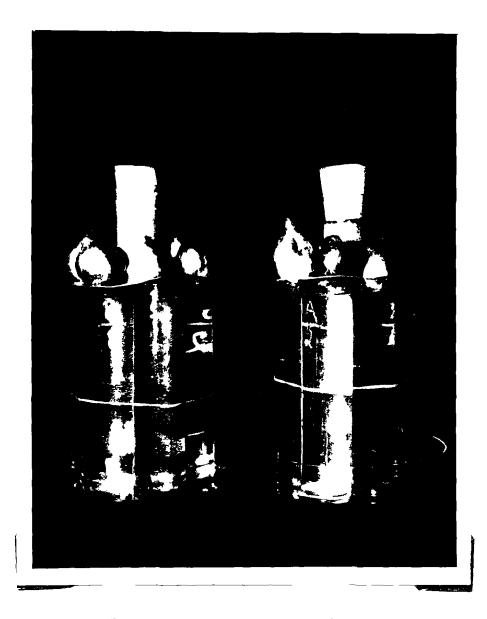
* Indicates that the results are significant at 5 per cent level.

TABLE - XIX

Table showing the inflorescences produced in the nonirradiated and irradiated group of onions

Treatment	Repli- cations	Weight groups	Number of inflorescences	Dates of flowering
No. Januari ta a	**		_	
Non-irradiated	II	6	1	1-1-1963
Irradiated 、	I	6	1	31-12-'63
	IV	6	2 ý	4-2-1963
		Ū	Ŭ Î	5-2-1963
	VI	4	2 Ø	8-2-1963
		-	- ŝ	9-2-1963
	~	6	1	4-2-1963
			Q Q	9-2-1963
	VIII	6	9 9 3 0 9 0	10-2-1963
			Q	12-2-1963
	IX	6	1	1 7- 2-1963
			0	1 7- 2-1963
		7	2 1	18-2-1963

- Fig. 1 Method adopted for rooting of onions while being irradiated.
 - A Ordinary sand (no irradiation).
 - B Monazite sand (irradiation).



B Fig. 1 A

Fig. 2 - Layout plan for laboratory experiment

- A Ordinary sand (no irradiation).
- B Monazite sand (irradiation).
 - I to IX Replications.
 - 2 to 7 Weight groups.

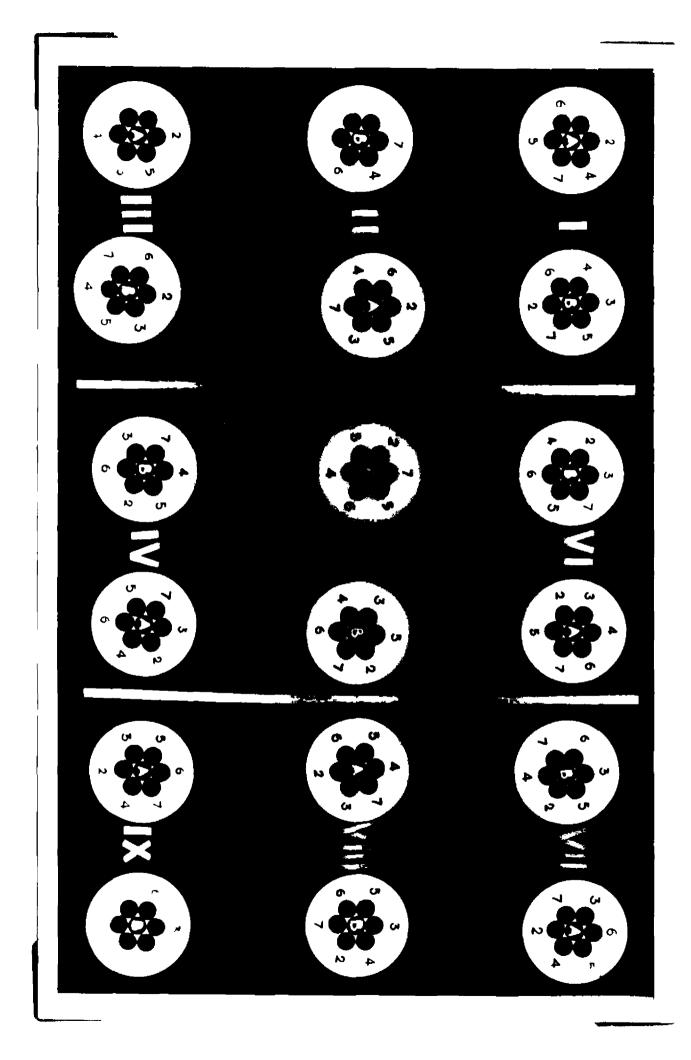


Fig. 3 - Comparison of root growth at 4 days after setting the experiment.

A - Ordinary sand (no irradiation).

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B - Monazite sand (irradiation).



Fig. 3 A



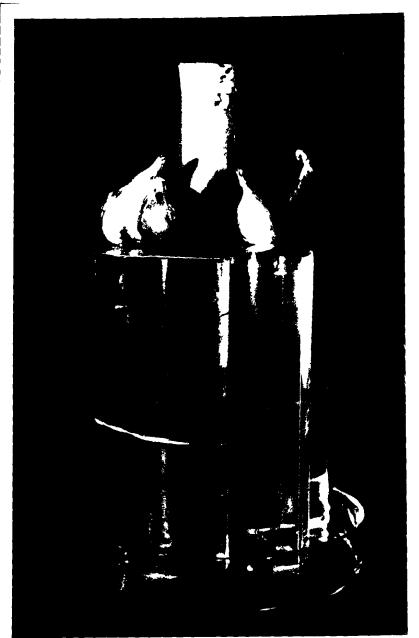


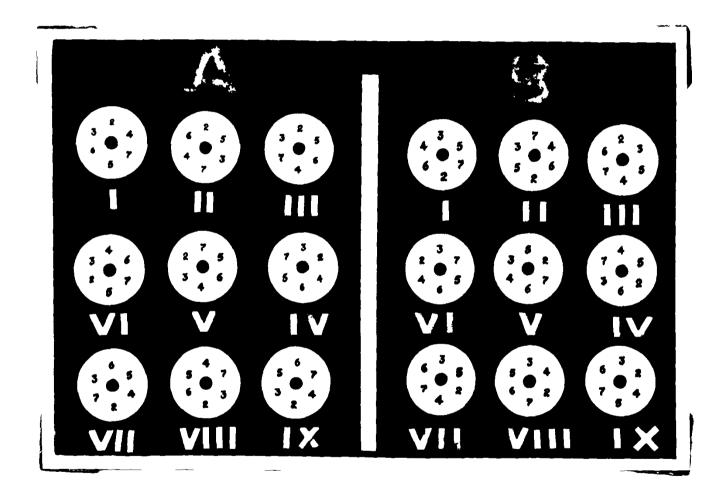
Fig. 3 B

Fig. 4 - Layout plan for field experiment.

A - Ordinary sand (no irradiation).

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- B Monazite sand (irradiation).
 - I to IX Replications.
 - 2 to 7 Weight groups.



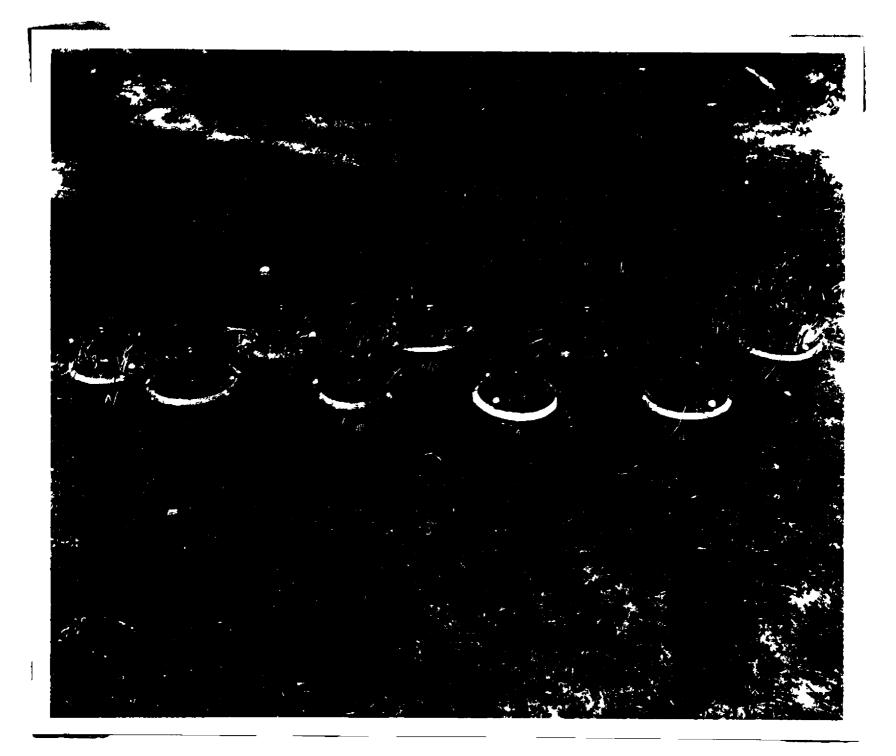
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Fig. 5 - Onion crop growing in the field.

- A Ordinary sand (no irradiation).
- B Monazite sand (irradiation).
 - I to IX Replications.





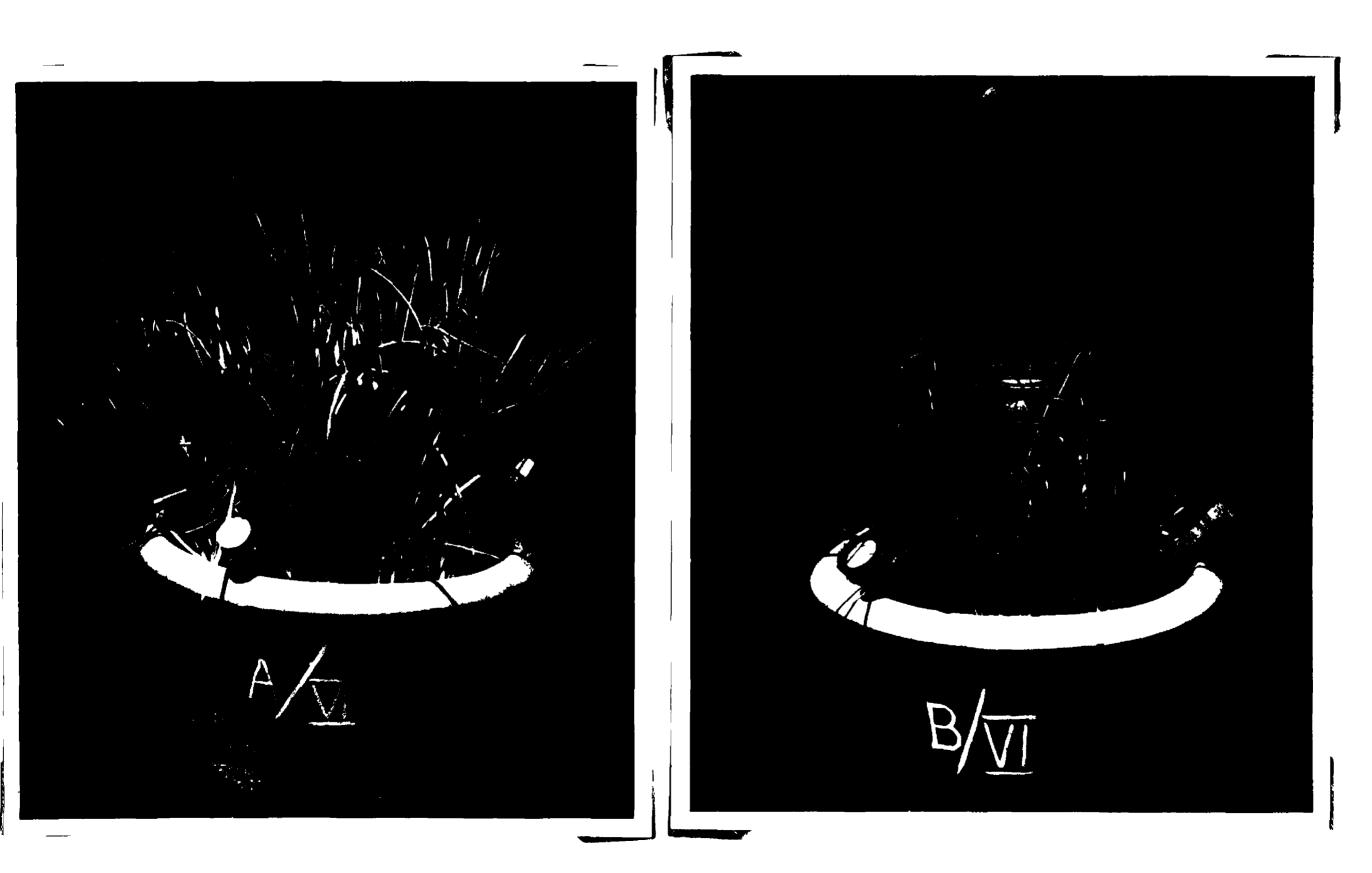


Fig. 7 - Bar diagram comparing the mean height of plants in non-irradiated and irradiated onions from second to eighth week.

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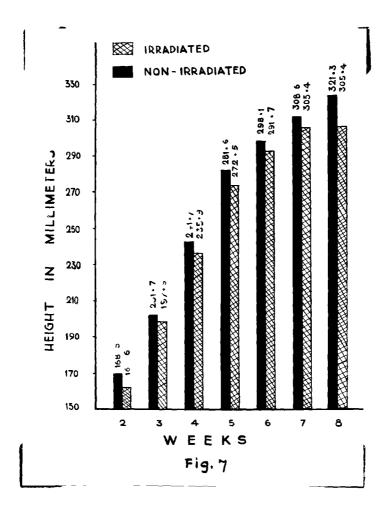
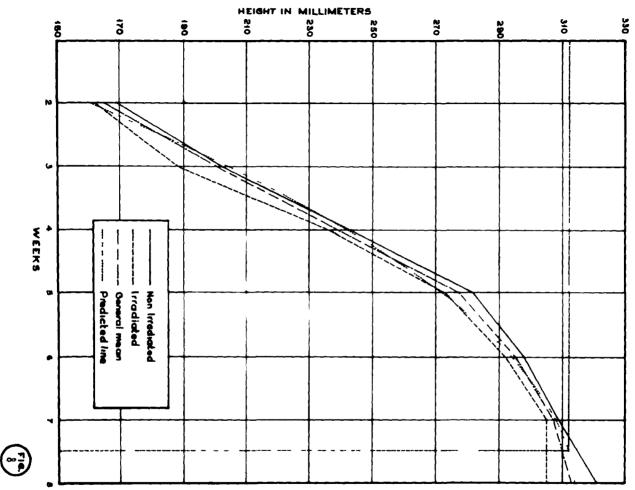


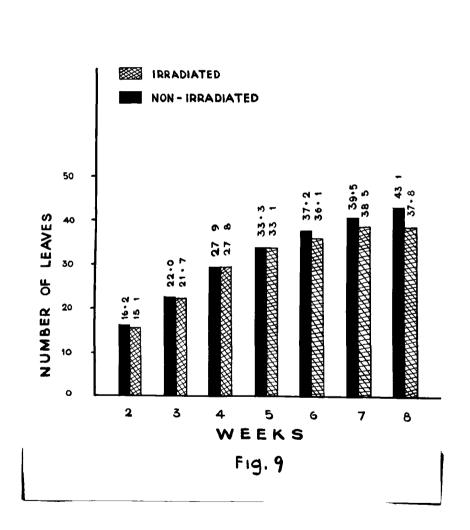
Fig. 8 - Graph representing the mean height of onions and predicted heights.



y=**48°8+65°6**×−3 **e**×²

Fig. 9 - Bar diagram comparing the mean number of leaves in non-irradiated and irradiated onions from second to eighth week.

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- Fig. 10 Microphotograph of cells from root tip of onions showing anaphase chromosomes. (x 840).
 - A Non-irradiated no breakage.
 - B Irradiated showing breakage.

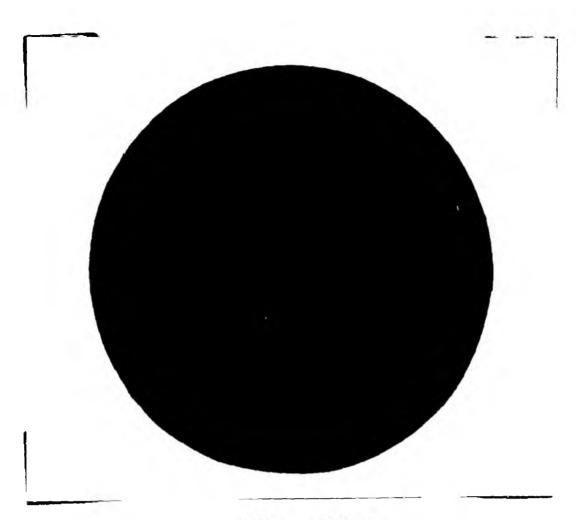


Fig. 10 A

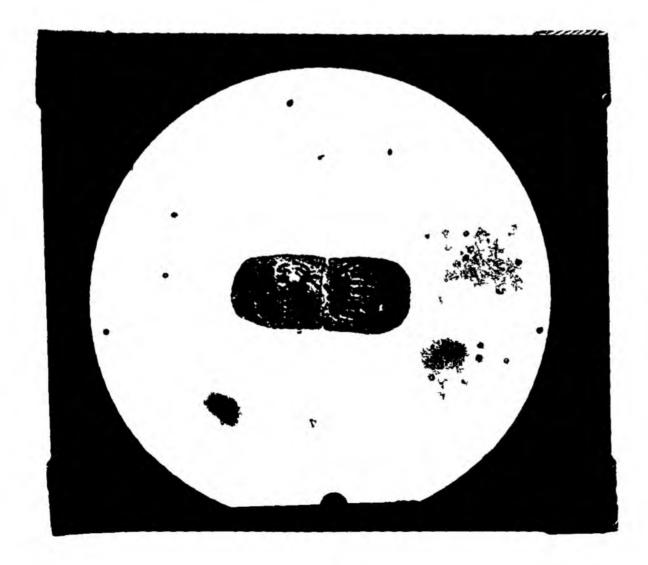


Fig. 10 B

Fig. 6 - Comparison of the flowering habit of the non-irradiated and irradiated onions.

A/VI - Non-irradiated -No inflorescence. B/VI - Irradiated -One inflorescence.

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