INVESTIGATIONS ON THE EFFECTS OF X-RAYS ON COW PEA (*Vigna Sinensis* L. SAVI.)

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide work carried out by Sri P.N. Ravindran Nair, 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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C O N T E N T S

INTRODUCTION

The use of ionizing radiations in medicine for various therapeutic and diagnostic purposes and in biology as an effective mutagen has stimulated the Scientists to investigate the mechanism of action of these radiations on living organisms. The wast development in the production of atomic weapons and the realization of the extent of damage to the living organism, caused by the radioactive fallout, emerged from the use of such weapons, greatly stimulated such studies. More than that the modern biologists and geneticists are probing at the ultimate secret of life or the fine structure of the chromosome, the gene, and their nucleoprotein components. It is of considerable interest that the use by geneticist of the knowledge and tools of radiobiology is making a significant contribution to our new knowledge of genetic fine structure.

Radiation genetics had its origin in the researches of Muller and Stadler almost thirtyfive years ago. They demonstrated that by means of X-rays it was possible to induce genetic changes in animals and plants. These researches really opened a new era for fundamental research in genetics. Soon after the above discoveries, it became evident that such induced mutations can profitably be employed in plant breeding programmes.

Plant breeding is controlled evolution. Two of the major factors in evolution - recombination and selection -

are extensively used by breeders and refined methods were developed during the first half of this century to exploit them. In the last thirty years, research has shown that mutations, the third major factor in evolution, offer an additional tool, which is potentially able to modify and improve cultivated plants in a way similar to conventional breeding methods. Induced mutation by ionizing radiation is playing a most important role in the present day breeding programme.

The nature of these induced mutations is a much debated topic which even now remains unsettled. In general there are two major schools of thought. The Muller school holds that the majority of the induced mutations represents intragenic mutations or true or gene mutations (Muller 1954). The second school represented by Stadler and coworkers, holds that induced variations are extragenic in origin, mostly lethal or sub-lethal and the non-lethal ones are probably a result of deletion of the components of a complex locus leaving at least one of the sub-units in-tact. (Stadler 1954).

The plant breeder is more interested in the practical utilization of these radiations to his advantage. The method of mutation induction as a plant breeding tool has been proved useful. Its usefulness was demonstrated first in Sweden through the brilliant researches of Nilson Ehle, Gustafsson and their co-workers, who demonstrated in cereals that higher yield, stiffer straw and earlier ripening types could be produced by radiation. Considerably later, its usefulness was demonstrated in the United States, shortly after World War II, in a comprehensive experiment of Gregory on Arachis hypogaea. Its usefulness in the transfer of disease resistance from wild relatives to cultivated plants was shown by Sears (1956) and Elliott (1958) in wheat and Konzak (1956) in oats.

Besides the utilization of X-rays to plant breeding programmes, it proved useful to solve many other biological problems. It has been shown that during the life of a plant some internal systems or cells are more easily modified by radiation than others. (Henshaw and Francis 1935). Further it has been shown that radio-sensitive systems may be modified by changes in the external or internal environment (Caldecott and Smith 1952; Mikaelson 1954). A study of these possible mechanisms of effects may help to solve some of the physiological problems of growth and differentiation.

In the present investigation an attempt is made to evaluate the morphological and cytogenetic effects of X-rays on the common cowpea (Vigna sinensis) . Cowpea is a prominent member of the pulse group, extensively used in the States of Kerala and Madras. The plant is naturally self fertilized and in which very little genetic variability exists. Due to this fact induction of genetic variability through artificial mutation seems profitable.

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R E V I E W O F L I T E R A T U R E

Since the discovery of X-rays by Rontgen in 1895 scores of scientists worked on the effects of these rays on plants and animals. With the discovery of Muller (1927) a special impetus was received for the mutation research. In the earlier days of research, the main interest was directed to the morphological and physiological changes induced by X-rays. But in more recent years the main interest of work has been directed to the understanding of the genetic effects. The possible utilization of X-rays in economic plant and animal breeding has received still more importance, A short review of the effects of X-rays on plants is given below.

MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF X-RAYS.

Effect of Germination.

Three years after the discovery of X-rays, Maldiney and Thouvenin (1898) found that the treatment of dormant seeds of *Convolvulus* and *Lepidium* hastened germination. Pfeiffer and Simmermacher (1915) reported that the germination of *Vica faba* was increased by short exposures to X-rays but lessened by long exposures. Contradictory to the above results Ancel (1924) in her experiments using hundreds of seeds, found that in no case germination was hastened by irradiation.

Kumar and Joshi (1939) reported the effect of

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X-irradiation on the seeds of Brassica juncea, Nicotiana tabacum and Pennisetum typhoides. They found that irradiation impaired germination in all cases. Krishnaswamy and Rengaswamy Ayyengar (1941) reported that in Pennisetum typhoides X-irradiation caused no reduction in germination percentage, but survival was reported to be very low in treated seeds.

Gustafsson and his co-workers published a series of papers on the effects of ionizing radiations on various crop plants. Gustafsson (1941) reported that different kinds of plants react dissimilarly to X-irradiation. Especially insensitive are the seeds of the cruciferous plants which in certain cases (for eg. white mustard) endure doses of 1,00,000r. and even more without any marked reduction in germination percentage and survival. On the other hand seeds of pea (Pisum sativum) are very sensitive; their maximum effective dose being 10,000-20,000r. The same applies to the seeds of sunflower and safflowers which cannot endure 10,000r.

Froier and Gustafsson (1944) reported that the larger the X-rayed seeds the more will they endure irradiation and that their germinability and sprouting ability were better than the smaller seeds. These authors also reported that the sprouting ability falls proportionately with the increase in dose.

Higher germination percentage was reported by Jacob (1949) in irradiated seeds of Jute (*Chorchorous* sp.) Survival was also reported to be higher in some varieties used by him. Caldecott *et al* (1954) reported reduction in germination and seedling survival after X-ray and neutron irradiation. Wohrmann (1955) found that in *Alopecurus pratensis* germination was very adversely affected by dosages between 18 and 26 kr; the effect varying in linear proportion to the dose of X-rays used. Giles (1956) found that doses below 2,000 r had no effect on germination percentage in seeds of *Solanum acaule*; and in excess of this the percentage of germination decreased with increased irradiation.

Ehrenberg (1955) reported increased germination percentage and survival with low doses of X-rays. But with increasing dose-rate. Similar results were obtained by Spencer and Cabanillas (1956) with *Indigofera endecaphylla* (trailing indigo). They found that the irradiation appeared to promote earlier germination and that the seedlings displayed no definite lethal effects. Contradictory to the above reports, Lesley and Lesley (1957) obtained considerable reduction in germination in irradiated tomato seeds. Matsura *et al* (1957) also reported a considerable reduction in the germination percentage of wheat seeds after irradiation with 20 kr X-rays and gamma rays.

Micke (1958 and 1961) observed that when the seeds of Melilotus albus were treated with X-rays ranging from 10-100 kr the germination capacity was unaffected, but lethality happened after about two weeks. Gottschalk and Scheibe (1960) working with plants belonging to leguminosae reported that the germinability of seeds was independent of X-ray dose. Similar reports came from Kundu et al (1961) in Chorchorus sp; Jan Sjodin (1962) working with Vicia faba, Katayama (1963) in Oryza sativa, Shastry and Ramiah (1961) in Oryza, Jain et al (1961) in chrysanthemum and many other workers. Swaminathan et al (1962) working with polyploid wheat also came to the conclusion that germinability of seeds was unaffected by low and moderate doses of X-rays.

GROWTH OF PLANTS.

Kornicke (1904) reported that in Brassica and Vicia X-ray treatment of seeds caused growth checking followed by an acceleration of growth which was transitory if the doses were not too high. The same author in another report (1915) stated that X-rays parallel other rays in showing growth retardation with stronger doses but growth stimulation with weaker doses. Miege and Coupe (1914) conducted irradiation experiments on Raphanus and Lepidium and reported a stimulating effect which manifested itself in increased weight of leaves of irradiated plants as well as an increase in the totalweight. Schwarz (1914) also found accelerated growth in Vicia faba with weak doses of X-rays.

Jungling (1920) observed that with light doses root growth was unaffected while shoot growth was increased.

Transitory acceleration of development has been observed by Altman *et al* (1923) in *Phaseolus vulgaris* irradiated with X-rays. According to them the stimulation dose varied with the stage of development. Ancel (1926) based on her extensive experiment concluded that the so called "stimulating effect" of X-rays on development of plants did not exist.

On the basis of experimental evidence obtained on twelve diffierent species of plants Schwartz *et al* (1923) denied the stimulating action of X-rays.

Geller (1924) after examining the results of experiments covering the period of 1910-1923, concluded that lower doses of X-rays caused acceleration of development while higher doses produced a depressing action. He believed that there was no definite dose for retarding or stimulating plant growth since the effect was dependent on so many factors.

Arntzen and Krebs (1925) working with peas found that with light doses a stimulating effect could be demonstrated for the first, and in some cases for the second 24 hour period of exposure. This was followed by retarded growth.

In treating dry and sprouting seeds of wheat and peas with various doses of X-rays, Koltsov and Koltsov (1925) did not observe any stable stimulating effect.

Revera (1920) considered increased development of aerial buds following irradiation as a stimulation. In the same year he reported that X-irradiation caused the retardation in the development of caster beans. Goodspeed and Olson (1928) stated that a marked accelaration of growth and development may result by appropriate X-ray doses. Shull and Mitchell (1933) concluded that stimulative effect may be obtained if appropriate conditions were employed. They demonstrated the accelerated development using 30-120r of filtered X-rays on seedlings of common wheat oats and sunflower. Earlier flowering was also reported by them.

There were a number of earlier reports indicating that no kind of stimulation was associated with X-rays but only retardation. Capizzaro (1926) reported temporary retardation of development occurring in proportion to the time of irradiation and the quality of rays used. Maisen and Mary (1928) reported marked inhibition of growth when seeds of Pisum sativum were exposed to X-rays. Glocker et al (1929) found retardation in growth of beanseedlings particularly with softer X-rays.

Moore and Haskins (1933) reported complete inhibition of terminal bud and extreme fasciation in cotton irradiated with high voltage X-rays. Johnson (1931) described excessive branching of stem of irradiated Helianthus and Lycopersicum. Patten and Wigoder (1939) subjected the seed of beans, mustard and barley to radiation but failed to get any marked stimulation. But Wort (1941) reported that wheat seeds irradiated with low doses of X-rays produced larger plants than the controls. Jacob (1949) reported "gigantic" plants in X-rayed *Chorchorus* Plants.

Gelin (1954) reported X-ray mutations in peas and vetches, and recorded "vitality mutants", ie. robust multi-branched types and "minus-mutants", such as dwarfness, chlorophyll defectives, inhibited flowering etc. These "minus-mutants" were reported to revert back to normal types in later generations. A gigas form was also reported by him. Bruns (1954) employed a dose of 1,000-20,000r. in *Trifolium pratense* and reported dwarf and gigas mutants.

Kuzin (1955), summarising the works of Soviet Scientists observed that data obtained under practically similar conditions are indicative both of a stimulating effect and the absence of a stable positive action. He reported increased root elongation in rye following seed irradiation with 100r .In radish and pea, the root length was reported to be significantly greater than the controls in 900r.irradiated groups. This stimulating action was reported to be absent in soft wheats. In another variety of wheat, Frolov (1936) found that radiation gave higher yield and earlier maturity. Kuzin (1955) reported that a radish variety irradiated with 1000 r. gave earlier ripening.

Giles (1956) reported that in Solanum acaule X-irradiation caused a reduction in seedling growth leading to dwarfing. He reported two plants in irradiated lot which flowered profusely, produced larger berries than the controls. A slight increase in height and weight was observed by Spencer and Cabanillas (1956) in Indigofera endecaphylla following X-irradiation. Yagyu and Morris (1957) found that in X-irradiated tomato, the size of the first two leaves showed a linear correlation with the dose. Beard (1958) found that in barley, maize, mustard and safflroweres the height was linearly related to the dose.

From an elaborate study Schwartz and Bay (1956) were able to establish the seedling height-dose curve, plotting the seedling height against dose. They found that at low levels there was the expected inverse relation, but a point was reached after which the original trend was reversed and a further increase in radiation dose resulted in an increase in seedling height. In an earlier paper Schwartz (1954) noted that at very high doses around 5,00,000 r , where the seedlings were turgid and looked perfectly normal, growth had ceased after about 5 days. Since no dividing cells was observed it was suggested that the growth was entirely due to the elongation of cells already present in the embryo.

Bose and Rao (1959) in an extensive work on the induced mutations in rice reported that the X_1 plants from seeds exposed up to 75,000 r were normal in appearance, but showed slow growth in the seedling stage. Shastry and Ramaih (1961) reported considerable reduction in height of the seedlings, the reduction being most pronounced beyond 22 kr. Premature tillering was noticed by them in certain dwarf plants.

Scossiroli et al (1961) reported X-ray experiments on Triticum where they observed that both in tetraploid and hexaploid wheats X-ray treatment reduced the length of roots significantly. Similarly, significant reduction in seedling length was also noticed by them in the treated series. They also recorded that the average length of internodes was shorter in treated plants than in the control. This led to the reduction in total plant height. Scarascia et al (1961) reported that in wheat the mean root and shoot length was a function of dose. They found that certain varieties were more sensitive to X-rays than others.

Micke (1961) reported a 78% reduction inheight of plants at flowering time after irradiation with 40,000 r irradiation with 5,000-10,000 rep of neutrons, the growth was stimulated. But no such stimulation was observed after X-irradiation.

Thus the published reports lead to no definite conclusions regarding the effect of X-rays on growth. In general it is agreed that at high doses growth shows an inverse relationship. Again it is influenced by many factors. The stimulating effect of X-rays reported by many authors has not yet been proved conclusively.

FERTILITY

The fertility of the X_1 plants has been studied by several authors with several plants. Gustafsson (1944) and Freisleben and Lein(1943) reported that the fertility in barley decreased with increasing dose. Similar result came from Mackey (1951) in barley. Wohrmann (1955) studied the pollen fertility in *Alopecurus pratensis* and also obtained dose proportionality both in regard to distribution on fertility classes and to mean fertility.

Contradictory to the above reports, Genter and Brown (1941) stated that in *Phaseolus vulgaris* there was no difference in pollen sterility in the control and treated plants. Zacharias (1956) irradiated soy-bean (*Glycine soja*) and obtained a difference of 5% between control and treatments but no difference being observed between different doses.Both Tedin and Hagberg (1952) and Hackbarth (1955) found no difference in fertility between the treated and control materials in *Lupinus*.

Chaudhuri and Das (1956) found that in X-ray treated *Sesamum orientale* the percentage of pollen sterility increased with the dosage only upto 40,000 r. In two varieties they found that the pollen sterility decreased with further increase indose, while in another variety they found that the percentage of pollen sterility increased with further increase in dose.

Bekendam (1961) showed that in rice the percentage of sterility increased according to the dose only up to a certain limit indicative of a saturation effect. Thus the fertility in 1,50,000 r irradiated lot was 58.2% and in 3,20,000 r irradiated lot it was 59.4%. This shows that the linear relationship will hold true only up to a certain dose limit.

Most of the workers agree to the conclusion that pollen sterility is linearly related to the dose. Evidences were also provided by Ehrenberg (1955) in barley, Beard _et al_ (1959) in barley and Kundu _et al_ (1961) in _Chorchorus_.

Ovule sterility was also reported to be proportionate to the dose. The sterility in the female side is usually less drastic than in the male side. Gustafsson (1927) recognised two classes of sterility. The first comprises such mutants that already had reduced viability in the vegetative stage and consequently poor seed setting and mutants that were wholly or partially sterile due to altered spike or flower structure. Gustafsson (1947) reported many such mutants in barley. Westergaard (1928) as well as Gustafsson (1947) reported a peculiar mutant in most of the spikelets were sterile except the top ones. Gustafeson, (1941) reported another mutant with repeatedly ramified ears with complete ovule sterility. Similar sterility types were also reported by Harlan and Pope (1922) and Smith (1939).

Smith (1939) reported, in Triticum monococcum an antherless mutant, where the anthers ceased development before or shortly after meiosis.

The second type of sterility distinguished by Gustafsson (1947) was, that arose as a result of disturbed meiosis, caused by recessive factors.Smith (1939) reported such sterility in theprogeny of X-rayed Triticum monococcum.

EFFECT OF PRE-SOAKING ON X-RAY SENSITIVITY.

Since Stadler's investigations (1928 b) it has been known that the mutation frequency rises considerably if the seeds are soaked in water before irradiation. Moore and Haskins (1933) noticed that presoaking of seeds before irradiation enhanced the X-ray sensitivity of cotton seeds. Kumar and Joshi (1939) reported that soaking of seeds increased the mutation rate and that soaked ones injured more than dry ones. The finding of the above workers has since been confirmed by several investigators (Gustafsson 1940, Wertz 1940, Kaplan1940) .

Gelin (1941) analysing the comparative effectiveness of X-rays on dry and soaked seeds found that seeds containing 10% water show 12.66% disturbed division, seeds with 15% water 27.99% and seeds soaked for 23 hrs in water or in0.01% heteroauxin solution 53.8% and 50.99% disturbances respectively.

Gustafsson (1947) reported that the seeds that were soaked in water before irradiation showed an inferior sprouting ability, a higher number of dead seedlings and a higher X_1 sterility than seeds irradiated in the dry condition. Similar reports also came from D' Amato and Gustafsson (1948) They found that certain mutations such as the "alboxantha" did not arise after an irradiation of dry seeds but they were common in hydrated seed series.

Mackey (1951) reported that with small incease in water the cytogenetic irregularities became more numerous and according to him this effect, at least to a large extent, was due to an increase in volume of the seed. He also found that if the seeds were pre-soaked only for a few hours the increased sensitivity was reversible when the seeds were dehydrated again. Nybom et al (1952) found that the X-ray sensitivity of pre-soaked germinating barley seeds was about 6-7 times greater than that of dry dormant seeds, thus suggesting some correlation between water content and X-ray sensitivity.

Caldecott (1954) conducted certain experiments to establish the relation between hydration and radiation effect. He pointed out that "if the role of water in the sensitizing of a system to X-irradiation, is in large part due to chemical events incited through radical production, it would seem reasonable to assume that in any biological system in which the water content could be varied over rather wide limits, the radio-sensitivity of the system would increase with increasing water content". Caldecott found that the water content of the seeds should be increased from 7% of their total weight in the unsoaked condition to about 20% in the soaked seeds, before there was an increase in their radio-sensitivity. Increasing the water content of the seeds above 20% resulted in the striking increase in radio sensitivity.

In another report Caldecott (1954) presents evidence for an inverse relationship between the water content of the seeds and their radio-sensitivity. This relationship was found to hold true for doses of 10,20,30,40 and 50 kr. Caldecott (1955) also showed that the sensitivity of barley seeds to X-rays as measured by the inhibition of seedling growth and the frequency of chromosomal aberrations, decreased as the water content in the embryo increased from about 4% to 8%.

Lefort (1954) found that dry dormant seed of *Lycopersicum esculentum* was about 4 to 5 times as resistant to X-irradiation as seeds previously soaked in water.

Hackbarth (1955) from his experiments on 3 species of Lupinus found that the soaking of seeds in water previous to irradiation with a dose of 10 kr. gave a considerably greater number of mutants than the dry seeds.

Lesley and Lesley (1957) subjected tomato seeds to X-rays in the dry and pre-soaked conditions. They found that the percentage of germinating seeds was lower in soaked seeds than in the dry seeds and also more irregular. Plants obtained from soaked irradiated seeds were slower in growth, more irregular in meiosis and partially male sterile.

Ehrenberg (1958) concluded that soaked seeds were more sensitive to X-rays than dormant seeds, stored under ordinary conditions. In an earlier paper (Ehrenberg et al 1953) it was also reported that with low dosages the pre-soaked seeds reached higher levels of mutability than the dormant ones. In one instance he found that with a dose of 625 r in barley, the mutation rate in soaked seeds was seven times higher than that in dormant seeds. It was also shown by the same author that in barley there was a conspicuous decrease in mutation rate of pre-soaked seeds as the dose increased. Sooner or later the mutation rate dropped down to the characteristic mutation rate of dormant seeds. With very high dosages the pre-soaked seeds gave low mutation rates often lower than dormant seeds.

Hansel and Zakovsky (1956) found that thetypes of mutants produced were different in soaked and dry irradiated seeds. This was in accordance with the previous finding of D' Amato and Gustafsson (1948).

Hackbarth (1955) observed that the increased radiosensitivity of pre-soaked seeds holds true only in the case of X-rays and that in meutron irradiation there was no such difference. Confirmatory reports on this also came from Caldecott (1957) and from Matsuo and Ando (1958).

Ehrenberg (1955) stated that when cells irradiated with the same X-ray dose at 7 and 20% moisture, approximately the same amount of energy are dissipated per cell in both cases. The radiation injury was, however, about 4 times greater in the former. Such inverse relationship between water content and X-ray sensitivity was also reported by Korah (1957).

Shastry and Ramiah (1961) found that pre-soaking of rice grains before irradiation almost doubled the mutation rate.

Kundu _et al_ (1961) working with Jute observed fewer abnormal plants in the soaked and irradiated seeds than in dry irradiated.

Thus the reports on the role of presoaking in the radiation sensitivity are contradictory. But it is generally accepted that the presence of water, especially when it contains dissolved oxygen, is conducive of mutagenecis. This is in line with the ideas originally suggested by Fricke (Fricke 1934, 1935, Fricke and Hart 1936; Fricke et al 1938) who regarded much of the biological as well as chemical influences of ionizing radiations as due to the formation of active oxygen containing groups (OH, H_2 O_2) from water and from oxygen dissolved in water.

CYTOGENETIC EFFECTS OF X-RAYS

The past quarter century has witnessed a tremendous amount of research dealing with the cytological effects of various radiations. The literature on the subject is voluminous. The frequencies and types of aberrations have proved to be effective yard sticks for the cytrological assay of radiation damage. These data, together with those from comparable genetical and physiological studies, have provided some idea as to how the individual cell may be killed or altered temporarily or permanently by radiation.

The first published report of the cytological effects of X-rays was that of Lopriore (1898) who found that X-ray treatment of Valisnaria spiralis produced an acceleration of protoplasmic streaming which was checked by longer exposures. Later Seckt (1902) demonstrated the same effect in Oxalis and Mimosa . Williams (1923) found that X-ray treatment caused changes in cytoplasm viscosity.

Jungling and Langendorf (1930) demonstrated changes in the rhythm of nuclear division after X-ray treatment of V. faba root tips. Patten (1929) and Nakagawa (1931) obtained considerable mitotic depression after X-ray irradiation.

X-ray effect on gross chromossomal changes.

Gross chromossomal aberrations are the main observable cytological effect of X-rays. It is now known that chromosome breaks are produced with a frequency simply proportionate to the total dosage of ionizing radiation applied. Muller (1954) states that "the freqnency of gross structural changes varies as a power of the dose higher than one when X-or Gramma-rays are applied in ordinary doses since with such irradiation the brokenends which unite are usually produced by independently arising breaks and the products, the structurally changed chromosomes therefore represents a concatenation of effects".

The primary genetic event in structural changes has been proved to be due to the breakage of the chromosome thread. This interpretation was first advocated by Levitsky and Araration (1931) on the basis of their studies on plants Crepis, Vicia and Secale and by Stadler (1932) on the basis of his results with maize. This has further been supported by McClintock (1932 , 1938, 1939) Muller and his co-workers (1937, 1938,1939, 1940) and by Sax (1939).

Lea and Catcheside (1942) working with Tradescantia found that chromatid breaks and isochromatid breaks were induced at the prophase stage, while chromosome breaks and chromosome interchanges induced at the resting stage.

In barley Mackey (1951) reported that the frequency of cells with fragments gave a linear relationship to the dose with a slope down at higher dosages due to a saturation effect. In Triticum vulgare it was shown by Wagner (1950) that the neighbourhood of the centromere and the ends of the chromosomes were much more susceptible to X-ray effect and that breakage taking place mostly in these regions.

In Allium cepa Rasch (1952) observed the following effects of X-rays: temporary inhibition of mitosis, abnormal nuclear divisions caused by all types of chromosonal aberrations, abnormal cell divisions and finally degeneration of the nuclei and cells which were severely damaged by the original treatment or had suffered subsequent disorientation. The aberration frequency was found to be directly related to the dose.

Davidson (1957) observed on Vicia faba interstitial union of chromatids, mostly at homologous and rarely at non-homologous points and terminal union of chromatids (point unions). Such point unions did not occur in a limited segment about the centromere. Yagayu and Morris (1957) reported that in X-ray irradiated seeds of tomato, the most observable chromosomal changes were the anaphase bridges and fragments, the frequency of which varied linearly with the dose. Such a linear relationship between dose and bridges and fragments was recently reported by Basu (1962) in root tips of jute after seed irradiation with X-rays.

Patil and Bora (1961) reported multi nuclei formation followed by X-ray irradiation of seeds of Arachis hypogaea . Lagging of chromosomes as a result of the inactivation of centromere was noted by Sparrow (1961). Misdivision of centromere has been observed by Haque (1953) . Nondisjunction was noticed by Haque (1953), Swanson (1956) and Yagayu and Morris (1957) .

Swanson (1957) and Sparrow (1961) reported chromosome stickness and clumping as an immediate result of irradiation with high doses.

Persistent nucleoli were observed in irradiated material by McClintock (1941),Sparrow (1961) and Evans (1961).

Pseudochiasmata in the somatic cells were reported by D'Amato (1954) and by Haque (1961) following irradiation.

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OBSERVATION ON SEGREGATING GENERATION (X_2)

An analysis of the X_2 population will give an idea as to what extent the employed doses are effective in producing mutations. From such analyses various mutations were reported by many authors in various plants.

Gustafsson (1947) stated that the mutation types observed in the segregating generation might be divided into three types.

1. Chlorophyll mutants
2. Sterility and lethality mutants of different types
3. Vital mutants.

The vital mutations may be either morphological or physiological in nature.

Gustafsson (1940) recorded various types of chlorophyll mutations in the segregating generation of X-rayed barley. He identified various mutant types such as 'Albina,' 'Xantha,' 'Virescens,' 'Chlorina' etc., Freislben and Lein(1943) and Froier (1946) also recorded different types of such mutations in barley.

Sjodin (1961) stated that chlorophyll mutations were comparatively rare in the segregating generation of leguminous plants. He found that in leguminous plants the 'viridis' type was the most common, which was characterised by different shades of green. In one case he obtained a segregation ratio of 19 normal: 6 albina from an X_1 plant.

Blixt (1961) reported a type of chlorophyll mutant in X_2 and X_3 of peas, which has been named as 'chlorotica variomaculata', characterised by patches of green and yellow spots.

Morphological mutations in X_2

Leaf Types.

Goodspeed (1928) reported crinkled and puckered leaves in segregating populations of irradiated tobacco. Horlachar and Killough (1931, 1932, 1933) obtained a forked leaf mutant which inherited as a simple recessive. Gustafsson (1947) stated that both broader and narrower leaved mutants were common in the X_2 and X_3 of irradiated barley. Narrow and small leaved mutants were also reported by Athwal (1961) in Cicer.

Sjodin (1961) described an interesting 'unifoliata' mutant in the X2 of Vicia faba with simple leaves instead of the normal trifoliate leaves. Such unifoliata mutants had also been noted by Scheibe and Gottschalk (1956) and Gottschalk (1958).

Growth habit.

In many cases changes in the pattern of growth occurs under the influence of radiation. Thus Wohrmann (1956) noted that X-rays induced trailing habit in non-trailing types of Alopecurus pratensis. Gelin (1954) obtained multi-branched robust mutants from the X_2 of irradiated Vicia sativa.

Butler (1954) reported two peculiar types of tomato plants called 'propeller' and 'rosette'.

Athwal (1961) reported bushy mutants in the X_2 of irradiated Cicer, characterised by very small internodes and closely packed leaves and leaf lets.

Narahari and Bora (1963) noted short culmed mutants in the second generation of irradiated rice.

Flower changes.

Goodspeed (1930) noted flower colour changes in the progenies of irradiated tobacco. Interesting flower colour changes were observed by Sjodin (1961) in the X_2 of Vicia faba .Such flower colour changes were also reported by Bruns (1954) in Trifolium and Mehlquist (1957) in Carnation and by Hoffmann and Zoschke (1955) in Linum.

Sjodin (1961) reported mutants characterised by short corolla tubes, abnormal petals and deformed stigmas in the X_2 of Vicia faba. Jain et al (1961) reported double flowered mutants in the second generationof Chrysanthemum. Menon (1963 - Oral communication) obtained mutant plants in cosmos with two whorls of ray florets instead of one.

Bhatia and Swaminathan (1963) noted multiple carpel flower mutation in the segregating generations of irradiated bread wheat. This character was reported to be recessive to the normal.

Jagathesan and Shastry (1963) observed twisted and divided style in the M_2 of irradiated _Gossypium hirsutum_ Swaminathan (1963) reported a mutant in _Gossypium_ in which the staminal column and the style developed separately.

Fruit characters.

Lechner (1959) reported changes in the position of fruits in _Vicia faba_. Sjodin (1961) found plants with hairless pods in X_2 of _Vicia faba_ instead of the normal hairy types. Long and narrow pods were also reported by him.

Gustaffsson (1947) described the famous erectoid mutants characterised by very compact ears and with projecting kernals. Swaminathan (1963) obtained awned wheat plants from the X_2 of an irradiated awnless variety. Rai (1958) described a mutant in _Brassica_ with narrow slender erect pods. Tipped grain mutants were obtained by Narahari and Bora (1963) in rice.

Seed characters.

Stadler (1931) obtained a large number of mutants in _Zea mays_ with changes in seed characters. Most of these affect the colour of seeds. Almost all of these were reported to be inherited as simple recessives. Zachow (1958) obtained mutant plants in the X_2 and X_3 of irradiated Lupinus luteus characterised by smaller seeds. This proved to be monogenic recessive in nature. Another mutant isolated by him had speckled seeds. Sjodin (1961) reported that most of the mutations induced in Vicia faba were concerning the seed coat colour. He obtained about 15 seed coat colour mutations from the X_2 of irradiated V. Faba. The most common change being from the normal yellowish to black. Some of the colour changes were also reported to be associated with other characters such as earliness, growth habit etc.

Nishimura and Kurakami (1952) and Campos and Espiritu (1960) reported seed colour mutations in rice.

Sterility mutations.

Sterility mutants were reported to be common in the segregating populations after X-ray treatment. Such plants were abnormal and usually failed to flower. Such mutants were reported by Gustafsson (1947) in barley and Sjodin(1961) in Vicia faba. Athwal (1961) reported that the sterile mutants obtained by him in Cicer has got luxuriant vegetative growth and remained green for a longer time. He reported two types of sterile mutants, in one, the flowering was normal but the anthers failed to dehise and the pistil deformed. In the other, the flower was highly abnormal with non functional stamens and anthers.

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MATERIALS AND METHODS

The present investigation was carried out in the Division of Agricultural Botany, Agricultural College and Research Institute,Vellayani, Trivandrum in the year 1963-64.

The material selected for study is an established variety of cowpea (Vigna sinensis) known as "African" This is an early variety, which takes about 70-90 days to maturity. The plant is bushy in habit, grains are red and small, Pure seed material of this variety was originally obtained from the Pulse Specialist, Agricultural College and Research Institute, Coimbatore, and was maintained in the Division of Agricultural Botany Agricultural College and Research Institute, Vellayani. The seeds were uniformly dried in the sun. Seeds of uniform size were selected for the investigation.

The X-ray irradiation was given at the Agricultural College and Research Institute, Coimbatore, by a Philips X-ray unit. The machine operated at 50 KVP and with 3 mA. of tube current without filter. The target distance was 12 cms. The dose delivered was 68 r/second.

The seedlot for irradiation was divided into two. One half was soaked in distilled water for 10 hours and the other half kept as dry seeds.

Both soaked and dry seeds were spread in cardboard boxes in a single layer and then exposed to different doses of X-rays. The doses employed were 1,000, 3,000, 5,000, 7,000, 9,000, 11,000, 13,000 and 15,000 r units. Two hundred seeds were irradiated for each treatment.

Thus there were eight doses and 16 treatments. Two controls, one soaked and the other dry, were taken for comparison. Table 1 shows the different treatments.

The irradiated seeds were sown the next day. A randomised block design, with four replications was used. Ten seeds were sown in each pot.

Along with the pot culture, another lot of hundred seeds from each treatment and control were sown in beds to study the germination percentage, survivalpercentage and to get a large population for obtaining informations on chlorophyll mutations, pollen sterility and other morphological changes.

For mitotic studies the treated and control seeds were germinated on moist filter paper in petridishes. When the roots attained about 2 cm. in length, the tips were cut and fixed in 3:1:1 alcohol acetic acid, chloroform mixture.

Out of the ten seeds sown in each treatment few seeds did not germinate and among the germinated ones few did not survive. It is known that lethality mainly occurs

T A B L E I

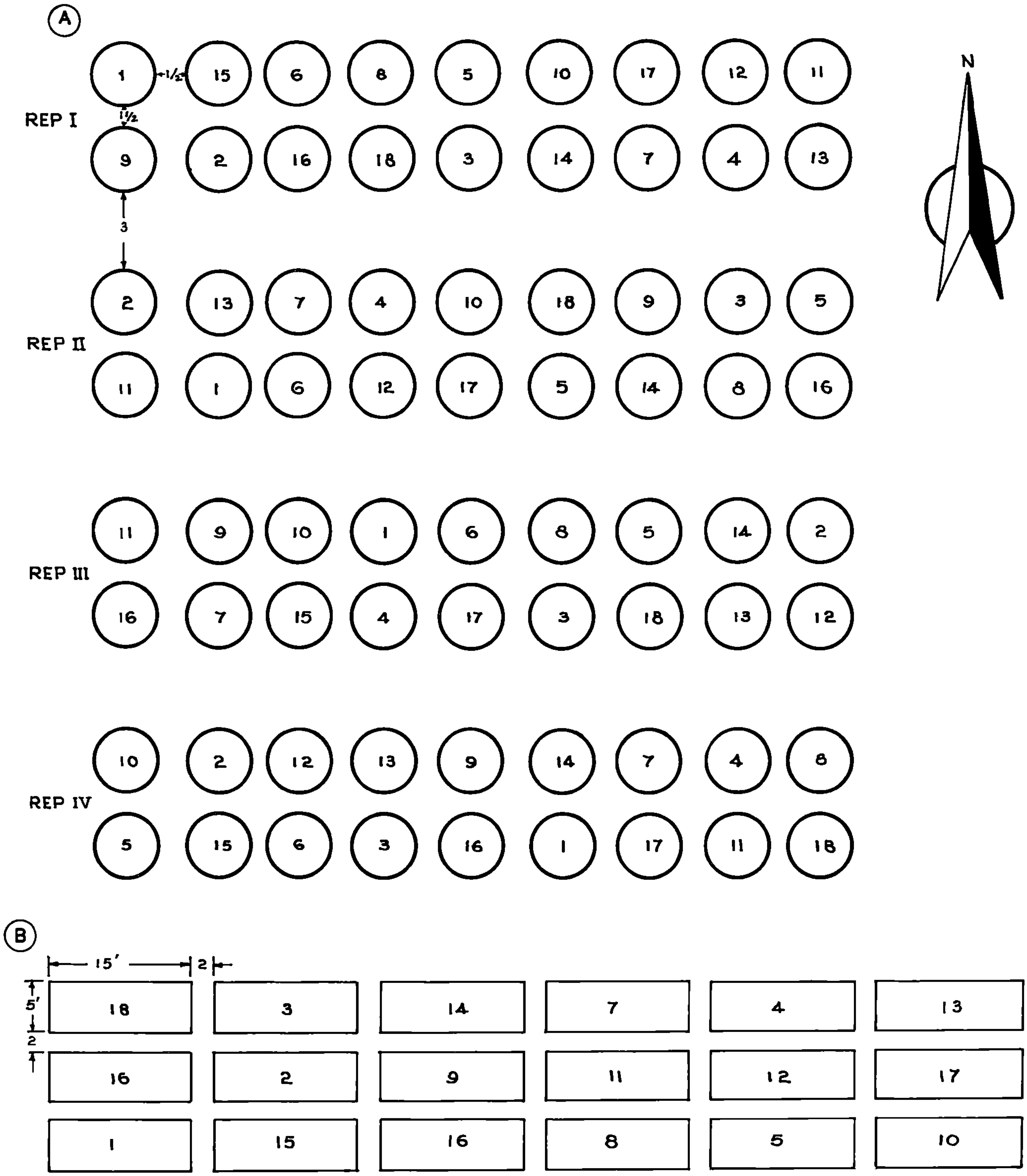
TREATMENTS EMPLOYED IN THE EXPERIMENT

Treatment No.	Treatments.
1	1,000 r Soaked
2	1,000 r Dry
3	3,000 r Soaked
4	3,000 r Dry
5	5,000 r Soaked
6	5,000 r Dry
7	7,000 r Soaked
8	7,000 r Dry
9	9,000 r Soaked
10	9,000 r Dry
11	11,000 r Soaked
12	11,000 r Dry
13	13,000 r Soaked
14	13,000 r Dry
15	15,000 r Soaked
16	15,000 r Dry
17	Control Soaked
18	Control Dry

Fig.1. Plan of lay-out of the experiment.

A. Pot culture

B. Field lay-out.



PLAN OF LAYOUT OF THE EXPERIMENT [X_1]

FIG 1

in the seedling stage. So to maintain uniformity excess plants were removed after three weeks, retaining six plants in each pot.

During the course of study the following observations were recorded.

1 Germinatinn percentage.

Germinatinn counts were taken from the 2nd day after sowing and continued until the 7th day. The final data were taken for analysis.

2. Percentage of survival.

Survival counts were taken at weekly intervals. The final data were taken for analysis.

3. Chlorophyll abnormalities.

Spotting of the first leaves with various intensities of green and yellow was the commonest chlorphyll abnormality. Such plants were counted and classified according to the intensity of spotting. The classification was done according to the system of Sjodin (1961), graded on a scale from 0-4. The grade '0' being assigned to the leaves which appeared normal, grade 4 was assigned to the most intensly spotted and generally deformed leaves and grades 1 - 3 being transitions between 0 and 4.

4. Plant height and growth.

Plant height was measured at weekly intervals from the second week after sowing. The data were analysed.

5. Pollen sterility studies.

Pollen sterility was studied from an entirely random sample. From each treatment ten plants were selected at random. From each plant one flower was taken. The pollen grains were stained with glycerine-acetocarmine. Ten fields were scored for sterile and fertile pollen grains from each slide at random. Thus hundred microscopic fields were scored for each treatment, from which the sterility percentage was calculated.

The acetocarmine staining method is not a measure of absolute fertility, but it gives a fairly good idea of the rate of sterility for comparative purposes.

6. Average flower production.

Average number of flowers produced in each treatment was counted and analysed.

7. Morphological Abnormalities.

Various morphological abnormalities observed were recorded and presented.

8. Average yield of pods.

Average yield of Pods in each treatment was taken and analysed.

9. Percentage of Fruit set.

From the total number of flowers produced and the total number of pods obtained, the percentage of fruit set was calculated for each treatment and analysed.

10. Average number of seeds per pod.

Average number of seeds per pod was obtained from random sample of hundred pods from each treatment and the data analysed.

11. Weight of 100 seeds.

This observation is included mainly to obtain information on the effect of X-rays on quantitative characters. A random sample of 1000 seeds was weighed and the data analysed.

Cytological studies.

Cowpea was found to be a difficult material for cytological analysis due to the small size of the chromosomes and the difficulty in obtaining good preparations. Both acetocarmine and aceto-orcein were found to be very unsatisfactory for the material. So the roots were stained in haematoxylin following Heidenhains method. The procedure consists in hydrolysing the root tips at 60°C in N.HCl for fifteen minutes, followed by mordanting in 4% Ferric - ammonium sulphate and staining in 0.5% haematoxylin solution. Squashing was done in 45% acetic acid.

From each treatment four slides were scored for normal and abnormal anaphases. The percentage of abnormal anaphases were calculated and analysed.

X_2 Generation.

From the X_1 generation of each treatment a random sample of 125 seeds were selected and sown in the field. Eventhough this was a very small population, it gave an idea on the types of mutations induced by X-rays. Chlorophyll mutations, and mutations affecting growth habit, flower colour, pod and seed characters were studied and presented.

Statistical technique.

Statistical technique consisted of Regression analysis, ie. finding out how far a given observation was related to the dose. To find out this, for each observation a suitable model (linear or quadratic) was fitted and the significance of regression was tested using the analysis of variance technique.

Among the observations,the total flower production was analysed using the analysis of variance technique for the randomised block design.

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EXPERIMENTAL RESULTS

The results of the present investigation on the morphological and cytological effects of X-rays on cowpea are given below

OBSERVATIONS ON THE X_1 GENERATION.

According to the definition adopted in the present work, plants raised from the X-rayed seeds form the X_1 generation . The X_1 plants are often heterozygous for induced genetic changes of various kinds. The immediate effects of X-rays are manifested in the X_1 generation.

Effect of X-rays on Germination.

In all treatments germination was noted in the second day after sowing in soaked series. In the controls complete germination was observed by the fourth day after sowing. By the eighth day after sowing almost all the seeds in all the treatments germinated. Delayed germination was noticed in certain cases. Such delayed germination was especially seen in the treatment No.13 (13 kr. soaked-irradiated) and 15 (15 kr. soaked-irradiated). Seven seeds in the treatment No.13 and six seeds in the treatment No.15 were found to germinate fifteen days after sowing.

The germination percentage in each treatment is given in Table II.

T A B L E II

Data on the % of germination, survival, pollen sterility, Total flower production and percentage of fruit-set.

Dose (kr units)	% of germina tion		% survival		% pollen sterility		Mean flower production		% of Fruit set	
	Dry	Soaked	Dry	Soaked	Dry	Soaked	Dry	Soaked	Dry	Soaked
Control	100	99	100	100	2.7	2.9	55	54	54.57	53.8
1	98	100	100	85.8	4.8	26.1	50	53	52.13	46.8
3	100	99	95	84.2	7.3	15.9	51	50	55.17	48.5
5	100	97	91	80	11.1	21.0	48	48	51.91	52.2
7	96	100	86.4	74	13.8	18.9	51	48	45.06	53.6
9	96	96	80.2	68.5	16.4	21.2	53	56	49.14	47.5
11	89	97	76.4	62.8	20.5	23.5	46	62	49.46	53.0
13	93	79	69.8	59.6	24.9	24.8	48	58	52.92	47.9
15	100	81	62	55.8	28.8	27.5	49	57	53.54	46.4

TABLE III

Analysis of variance for testing the significance of regression.

Percentage of Germination. (Soaked series)

Linear model ($y = 1.33x + 86.32$)

Source	Sum of squares	Degrees of freedom	Variance	F-ratio
Total	545.556	8		
Due to Regression	379.346	1	379.346	15.9**
Deviation from regression	166.21	7	23.74	

** Significant at 1% level $r^2 = 0.69$

T A B L E IV

Analysis of variance for testing the significance of regression. Germination percentage (Dry series)

Linear model ($y = 0.342x + 92.679$)

Source	Sum of squares	Degrees of freedom	Variance	F-ratio
Total	108.889	8		
Due to regression	32.2738	1	32.2738	3.14
Deviation from regression	72.6152	7	10.3736	

The table revealed that the germination of seeds was not affected by the X-ray treatment. The regression analysis was carried out, the result of which is given in tables III and IV.

The low F-ratio showed that in the dry series the regression was not significant which indicated that there was no relationship between dose and the percentage of germination.

In the soaked series the F-ratio was significant. This showed that there was a linear relationship between dose and the germination percentage. Germination percentage was thus inversely related to the dose. The calculated r^2 was 0.69. So the linear model fitted ($y = 1.33\ x + 86.32$) was a satisfactory fit for the data.

The regression equations and the regression lines are presented in figure 2.

Survival of plants.

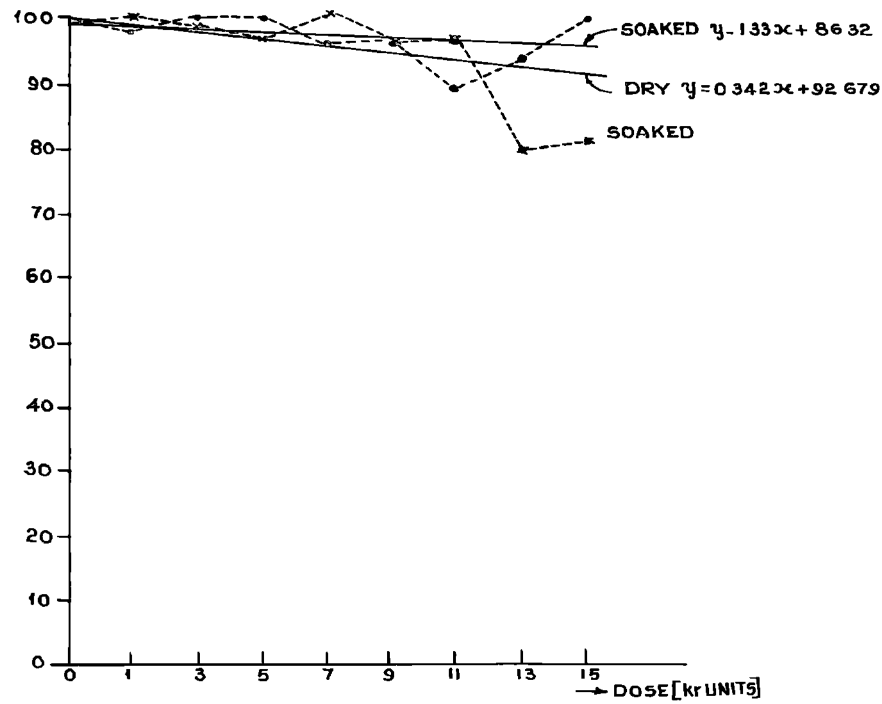
Survival studies were conducted at the time of the harvest. Only bearing plants were accounted as survivals. The percentage of survival in each treatment is presented in Table II.

To the data on dry and soaked series linear models were fitted and when tested these linear models

Fig. 2. Regression lines and regression equation for the percentage of germination.

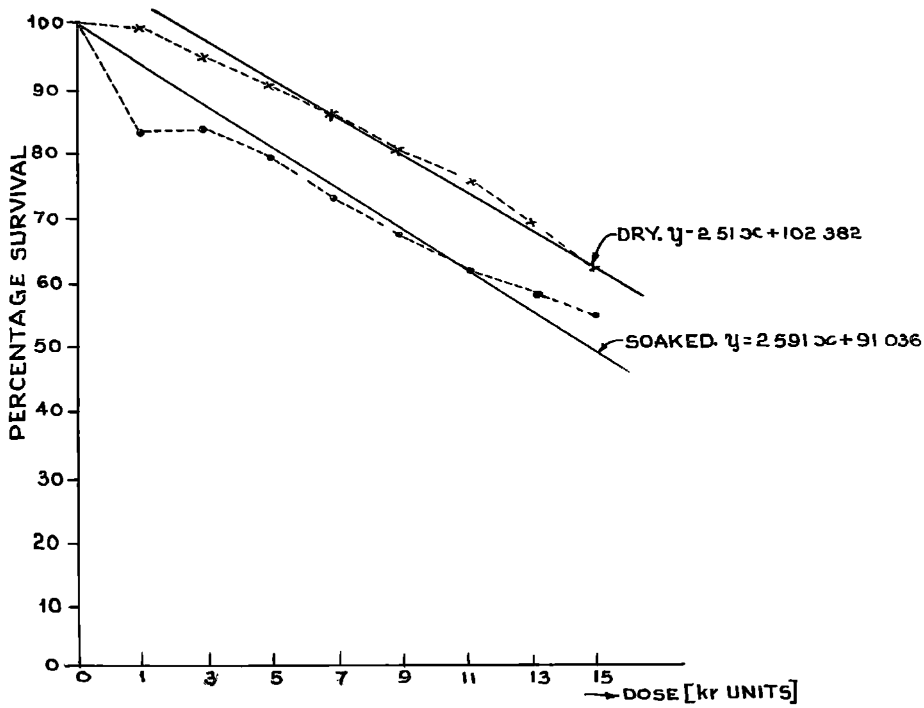
Fig. 3. Regression lines and regression equation for the percentage of survival.

FIG 2



PERCENTAGE OF GERMINATION

FIG·3



PERCENTAGE OF SURVIVAL

were found to be good fit. Tables V and VI showed the results of the regression analysis. The F-ratios obtained were highly significant in both cases. This showed that the percentage of survival was directly dependant on the dose and that there was a decrease in the percentage of survival as the dose increased The calculated r^2 was very high (0.98 and 0.92 respectively for dry and soaked) indicating the linear models were good fit for the data.

The regression equations obtained are given below.

Soaked series $y = 2.591x + 91.036$

Dry series $y = -2.51x + 102.382$

The regression lines are presented in fig. 2.

The data showed that the minimum percentage of survival was in the 15 kr. soaked irradiated, where the percentage of survival was only 55.8. A comparison between the dry and soaked series brought out the differences between them and the effect of pre-soaking on X-ray sensitivity. In the soaked series, the lowest dose (1 kr.) gave only 83.8% survival while the same dose in the dry series produced no effect on survival percentage. In all doses used it was found that the survival percentage was lower in the soaked series than in the dry series.

Observations indicated that lethalities mainly

T A B L E V

Analysis of variance table for testing the significance of regression

Percentage of survival (Dry series)

Linear model($y = 2.51x + 102.382$)

Source	Sum of squares	Degrees of freedom	Variance	F-ratio
Total	1443	8		
Due to regression	1418.30	1	1418.30	458.96**
Deviation from regression	24.74	7	3.09	

** Significant at 0.1% level $r^2 = 0.98$

TABLE VI

Analysis of variance for testing the significance of regression - Percentage of survival (soaked series)

Linear model ($y = 2.591x + 91.036$)

Source	Sum of squares	Df	Variance	F- ratio
Total	1629.4489	8		
Due to regression	1514.0667	1	1514.0667	91.27**
Deviation from regression	115.4213	7	16.4887	

** Significant at 0.1% level $r^2 = 0.92$

occurred at the very early seedling stage. Lethal seedings were usually pale, stops growth and began to wilt during the first week after germination. Seedling counts after two weeks showed that about 95% of the lethalities occurred during the first two weeks.

Seedling abnormalities

Cotyledons and the first leaf.

Cotyledons and the first leaves were normal in almost all seedlings. Unequal cotyledons were noted in six plants. among the treated plants. One plant each was observed in treatments 3, 7, 9, 13 and 15 kr soaked seed irradiated series and one plant in 9 kr dry irradiated. In all these cases one of the cotyledons was found to be very small and rudimentary, while the other very large.

Crinkling of the first leaves was observed in many seedlings. The time of emergence of the first leaves was also unaffected in most cases. The first leaves emerged in most of the plants, the day following the full emergence of the seedling from the soil. ie. 5 to 6 days after sowing on an average. Vatiations in the time of emergence was noted in fifteen seedlings - 3 plants in 1 kr.soaked, 1 plant in 3 kr soaked, 2 plants in 9 kr soaked, 1 plant in 13 kr soaked, 2 plants in 15 kr soaked, 4 plants in 15 kr dry and 2 plants in 11 kr dry irradiated series.

These seedlings on an average took 7 to 10 days for the appearance of the first two leaves after the plumule had emerged from the soil fully ie. about 12-15 days after sowing.

Chlorophyll variations.

Chlorophyll variations were the commonest type of variation observed in the seedlings. Chlorophyll variation included chlorophyll deficient spots, consisted of unevenly distributed groups of yellow and yellowish green patches of cells. The spots occurred most frequently in first and second leaves. Uniformly yellow seedlings were also noted.

The intensity of spotting was different in different plants. For convenience the plants had been graded in a five graded scale 0-4. The frequency of plants with different grades of spotting is given in Table VII.

The table revealed that the frequency of chlorophyll abnormalities were much more common in the soaked series than in the dry series and that there was an increase in the number of abnormal plants as the dose increased. For testing this dependance a linear model was fitted to the data and it was found that this linear model ($y = 1.26x + 2.34$) was a good fit. The F-ratio obtained was significant at 0.1% level of probability. The calculated $r^2 = 0.94$, indicated that the linear model was a good fit. So there existed a linear relationship

T A B L E   VII

Types and Frequency of Chlorophyll abnormalities

Dose (kr units)	Total No. of Plants	No. of plants with various spotting grades.					Total No. of chlorophyll variants	% of chlorophyll variants.
		0	1	2	3	4		
1 kr (s)	140	132	4	2	1	1	8	5.7
1 kr (D)	140	138	2	-	-	-	2	1.4
3 kr (S)	139	126	5	4	2	2	13	9.3
3 kr (D)	139	136	2	1	-	-	3	2.1
5 kr (S)	136	124	6	3	2	1	12	8.8
5 kr (D)	140	138	2	-	-	-	2	1.4
7 kr (S)	140	125	8	2	2	3	15	10.7
7 kr (D)	136	132	3	1	-	-	4	2.9
9 kr (S)	136	118	9	4	2	3	18	13.2
9 kr (D)	136	131	3	1	1	-	5	3.6
11 kr (S)	135	115	10	4	4	2	20	14.8
11 kr (D)	129	124	2	2	1	-	5	3.8
13 kr (S)	108	87	3	5	9	4	21	19.4
13 kr (D)	133	126	2	3	1	1	7	5.2
15 kr (S)	120	93	5	6	7	9	27	22.5
15 kr (D)	140	129	4	3	2	2	11	8.5
Control (S)	139	-	-	-	-	-	-	0
(D)	140	-	-	-	-	-	-	0

S : Soaked   D : Dry

T A B L E VIII

Analysis of variance for testing the significance of regression - Chlorophyll abnormalities (soaked series)

Linear model ($y = 1.26x + 2.34$)

Source	Sum of squares	Degrees of freedom	Variance	F-ratio
Total	374.37	8		
Due to regression	353.43	1	353.43	118.204**
Deviation from regression	20.44	7	2.99	

** Significant at 1% level. $r^2 = 0.94$.

TABLE IX

Analysis of variance for testing the regression.
Chlorophyll abnormalities
(Dry series)
Linear model $(y = 0.433x + 0.243)$

Source	Sum of squares	Df.	Variance	F-ratio
Total	49.896	8		
Due to regression	33.7085	1	33.7085	14.6**
Deviation from regression	16.1875	7	2.3125	

** Significant at 1% level. $r^2 = 0.57$

between dose and the frequency of chlorophyll spotting.

Such a linear relationship was less evident in the dry series than in the soaked series. The calculated F-ratio was significant at 1% level. The calculated $r^2 \doteq 0.67$ indicated that the linear model ($y = 0.433x + 0.243$) was a satisfactory fit. But among the two series the soaked series gave better fit to the linear model.

As stated earlier, the chlorophyll spotting was evidently seen only in first and second leaves. The spots gradually decreased in frequency and are completely absent from fifth leaf onwards. Most of these plants survived and a few failed to flower and set fruits.

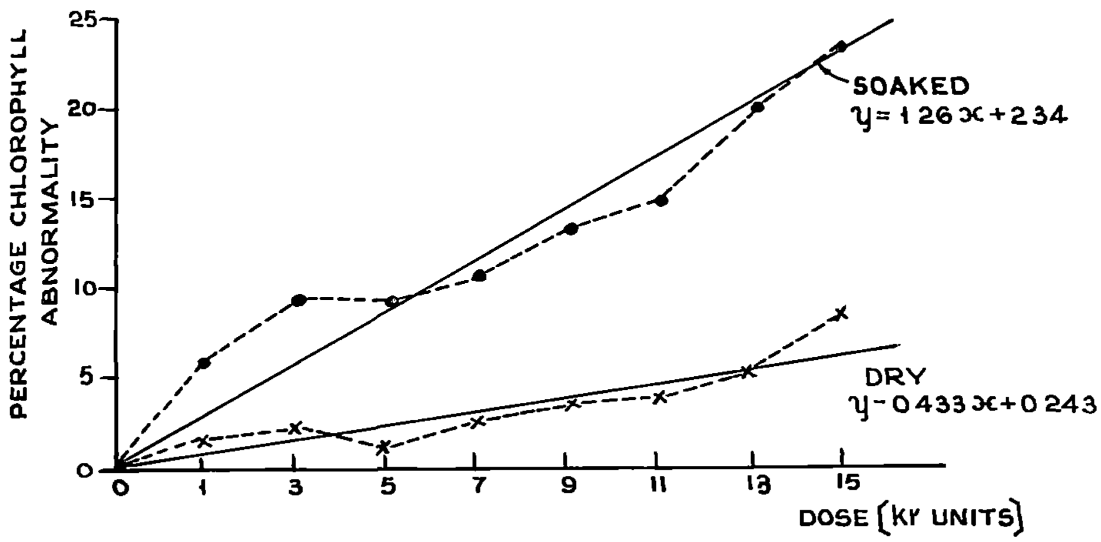
<u>Lethal 'Xantha' mutants</u>.

In the treated population 9 xantha mutants (completely yellow seedlings) were noticed, of which four were in 15 kr.-soaked treated seeds and 5 in 13 kr-soaked treated seeds. Out of the nine seedlings 6 were lethal, and one survived, which appeared abnormal and crinkled. This plant failed to flower. In the rest of the two seedlings the yellow leaves dropped off and after a few days new green leaves appeared. These plants were also abnormal and dwarfish.

Fig. 4. Regression lines and regression equation for the percentage of [illegible] chlorophyll abnormality.

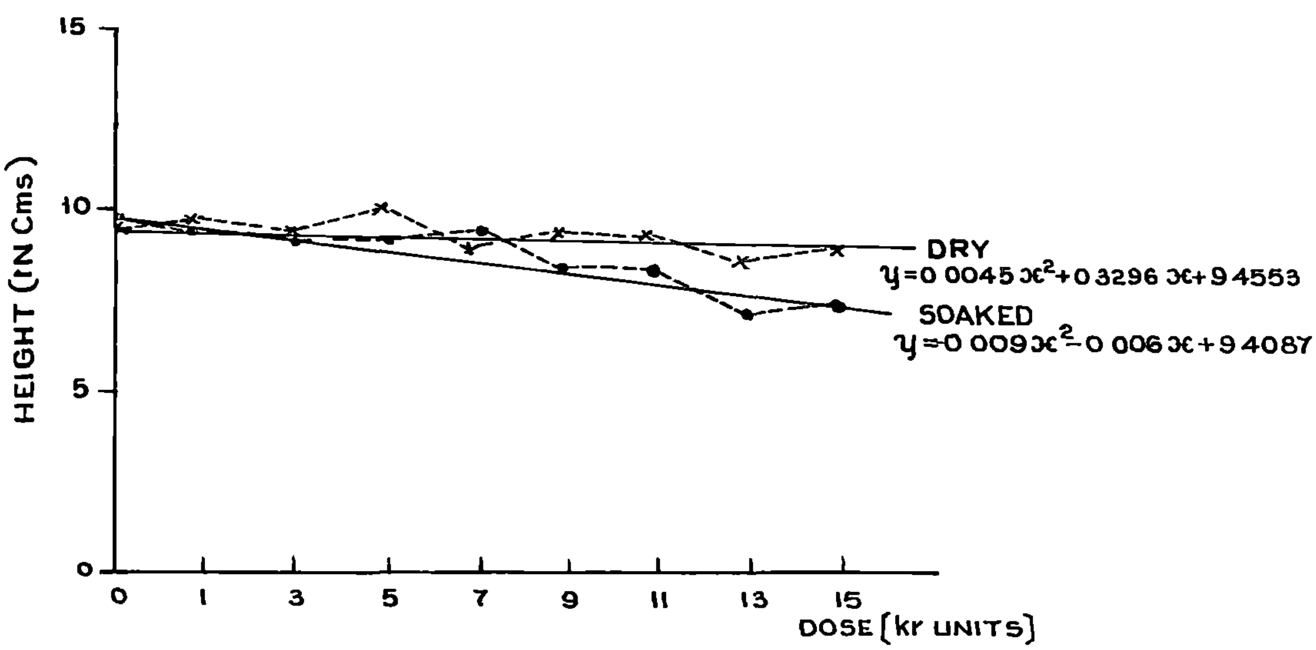
Fig. 5. Regression lines and regression equations for the height of seedlings (After two weeks)

FIG 4



PERCENTAGE OF CHLOROPHYLL ABNORMALITY

FIG 5



HEIGHT OF SEEDLINGS (AFTER 2 WEEKS)

Leaf Abnormalities.

Two plants, one each in 13 kr soaked irradiated and 15 kr dry irradiated, produced bifoliate leaves upto the fourth leaf stage. But from the fifth leaf onwards normal trifoliate leaves were produced.

Two plants, one each in 13 kr soaked treated and 3 kr dry treated produced simple leaves in the seedling stage. In both cases the leaves had only the terminal leaflet, the lateral leaf lets appeared as very rudimentary structures. This character also disappear later.

GROWTH OF THE SEEDLINGS

The height of plants was studied for getting information on the effect of X-rays on this complex character. The first height measurement was taken a fortnight after sowing. Tables 13 and 14 represent the results of the regression analyses.

The data on height in the dry series revealed that there was little difference in seedling stage. Both linear and quadratic models were found to be not significant. The regression analysis gave an F-ratio 2.22 which was not significant. This showed that X-rays caused neither depressing nor stimulating effect on seedling height.

But in the soaked series the regression analysis

TABLE X

Analysis of variance for testing the significance of regression - Seedling height (dry series)

Quadratic model ($y = 0.0045x^2 + 0.3296x + 9.4553$)

Source	Sum of squares	Df	Variance	F-ratio
Total	0.7830	8		
Due to regression	0.33332	2	0.16666	2.22
Deviation from regression	0.449687	6	0.074948	

T A B L E XI

Analysis of variance for testing the significance of regression - seedling height (Soaked series)

Quadratic model $y = -0.009\ x^2 - 0.0006x + 0.4087$

Source	Sum of squares	Df	Variance	F-ratio
Total	5.4053	8		
Due to regression	4.7539	2	2.3769	21.88**
Deviation from regression	0.6514	6	0.1086	

** Significant at 1% level

$r^2 = 0.87$

gave an F-ratio 21.88 which was significant at 1% level of probability. The linear model was found to be not significant. So there was no strict linear relationship between treatment and seedling height i.e. the height of the seedlings neither increased nor decreased linearly with the increase in dose. But the quadratic model was found to be significant. The model fitted was $y = -0.009x^2 - 0.006x + 9.4087$. The calculated $r^2 = 0.87$ which showed that the quadratic model was a good fit.

Height measurements in the subsequent weeks also revealed the same tendency as manifested by the first measurement. In the third week, in the dry series control gave an average height of 15.95 cms. In the 5000 r irradiated plants the height was 16.37 cms, and in the highest dose (15,000 r) the height found to be 15.38 cms.

In the soaked series, decrease in height was found in 13 kr and 15 kr irradiated lots.

Height measurements in the 7th week revealed the fact that the decrease in height in the 13 and 15 kr soaked series was transient and expressed only in the seedling stages. In the 7th week, it was found that the controls gave a height of 70.86 cms, while in the 13 kr soaked series the height was found to be 72.43 cms and in the 15 kr the height was 77.25 cms. This increase

T A B L E XII

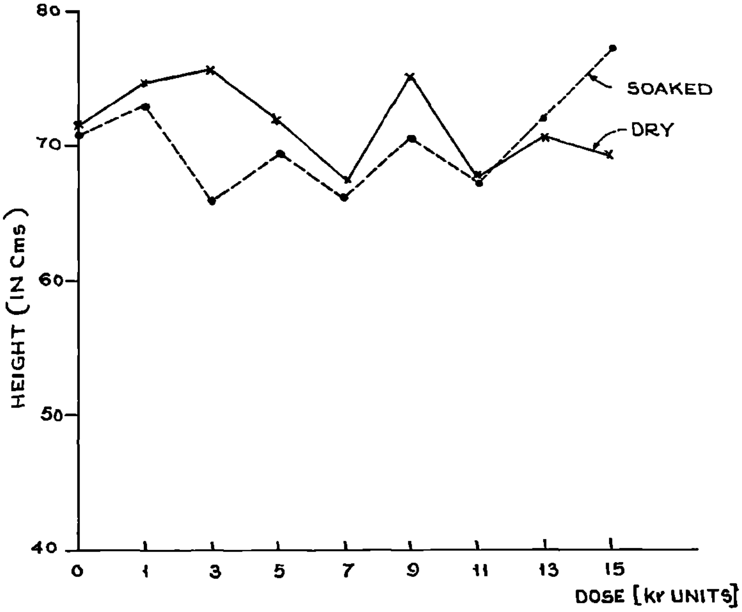
Data on weekly height measurements

Dose (kr units)	2nd week Dry	2nd week Soaked	3rd week Dry	3rd week Soaked	4th week Dry	4th week Soaked	5th week Dry	5th week Soaked	6th week Dry	6th week Soaked	7th week Dry	7th week Soaked
0	9.31	9.51	15.95	15.34	22.22	22.32	31.01	31.84	60.18	61.54	71.53	70.86
1	9.56	9.25	15.89	15.32	22.64	22.22	31.99	32.51	54.23	62.46	74.75	73.07
3	9.45	9.27	15.98	14.91	22.36	21.75	33.73	29.62	60.99	54.24	75.57	65.75
5	10.01	9.08	16.37	14.98	23.71	22.03	33.91	31.41	57.56	56.73	72.07	69.66
7	9.14	9.47	15.89	15.75	22.85	22.66	32.30	31.56	56.95	54.13	67.95	66.15
9	9.29	8.28	15.54	13.16	23.13	19.73	32.54	28.60	57.55	52.38	75.11	70.76
11	9.35	8.41	15.63	12.83	22.22	19.18	31.91	28.49	58.04	50.85	67.83	67.02
13	8.95	7.43	15.95	11.02	22.10	17.41	32.16	26.98	58.55	51.25	70.69	72.43
15	9.06	7.47	15.38	11.57	22.37	18.47	32.87	28.47	55.65	54.75	89.25	77.25

Fig. 6. Graph showing the final height of plants (After 7 weeks)

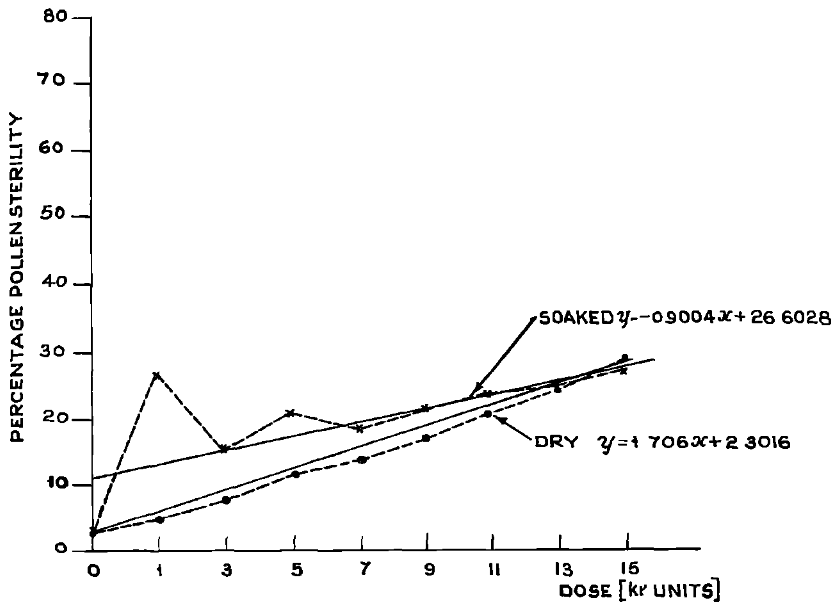
Fig. 7. Regression equation and regression line for the percentage of pollen sterility.

FIG. 6



FINAL HEIGHT OF PLANTS

FIG. 7



PERCENTAGE OF POLLEN STERILITY

T A B L E XIII

Analysis of variance for testing the significance of regression . Pollen sterility (dry series)

Linear model ($y = 1.706\ x + 2.2016$)

Source	Sum of Squares	Df	Variance	F-ratio
Total	658.83	8		
Due to regression	654.5122	1	654.5122	1061.15**
Deviation from regression	4.3178	7	0.6168	

** Significant at 0.1% level

$r^2 \simeq 0.99$

T A B L E XIV

Analysis of variance for testing the significance of regression . pollen sterility - (soaked series)

Linear model ($y = -9004 x + 26.6028$)

Source	Sum of squares	Df	Variance	F-ratio
Total	1441.26	8		
Due to regression	817.51	1	817.51	9.18*
Deviation from regression	623.75	7	89.107	

* Significant at 5% level. $r^2 = 0.56$

was not statistically significant.

The weekly height measurements are given in Table XII.

POLLEN STERILITY

The percentage of pollen sterility was calculated from an entirely random sample. The calculated percentage of pollen sterility is given in Table No. II.

From the very data it was understood that the percentage of pollen sterility was highly correlated with the dose. This was especially clear in the dry series. There was a linear increase in the percentage of pollen sterility as the dose increased.

The regression analysis of the data were given in Tables XII and XIII. In the dry series it was found that the regression was highly significant at 0.1% level and the calculated $r^2 = 0.99$ showed that the linear model fitted ($y = 1.706x + 2.3916$) was a very good fit for the data. In the case of soaked series, though the regression was significant, the linear model fitted ($y = -0.9004x + 26.6028$) was found to be not a good fit eventhough it was a satisfactory one. The r^2 was only 0.56 which indicated that the linear model was just satisfactory. In the soaked series it could be visualized that there was a rapid increase in the pollen sterility in the lowest dose namely 1 kr. The percentage fell down in the following doses of three and 5 kr and reached the minimum in 7 kr. Then again it increased linearly from 9 kr onwards and reached the maximum in the highest dose namely 15 kr. The data suggested that the pollen sterility was highest when low and very high doses were employed but was lower in the medium doses. Fig 7 gives the regression lines for the data.

FLOWER PRODUCTION

The analysis of variance for total flower production is given in Table No. XIV.

The analysis of variance showed that there was no significant difference in flower production between control and the treatment, thereby indicating that the treatment of X-rays has no effect in flower production. Again there was no significant differences between soaked and dry series in the production of flowers.

The variance table also revealed that the flower production was significantly differing between the doses employed.

When the doses were ranked it was found that the 9 kr-soaked was significantly superior in the flower production with mean number of 55 flowers. Doses 15 and 13

TABLE XV

Analysis of variance for
Total Flower Production

Source	Summ of squares	Df	Variance	F-Ratio
Total	96208.95	71		
Block	11133.83	3	3711.27	2.86*
Treatments	29174.70	17	1716.16	1.32
Treatment Vs Control	12441.84	1	1244.184	0.96
Soaked Vs dry	23697.56	1	23697.56	1.05
Between Doses	42272.94	7	6038.97	4.66**
Error	66000.42	51	1294.12	

* Significant at 5% level

** Significant at 1% level

R A N K I N G

Rank	Doses (kr units)	Mean No. of Flowers	Successive differences	Critical difference
1	9 kr	658.50		
			21.25	
2	15 kr	637.25		
			4.70	
3	13 kr	632.50		17.89
			12.25	
4	1 kr	620.25		
			13.25	
5	3 kr	607.00		
			15.75	
6	7 kr	591.25		
			2.50	
7	11 kr	588.75		
			9.50	
8	5 kr	579.25		

Conclusion 9, 15, 13, 1, 3, 7, 11, 5.

kr gave significantly greater flower production than 3, 7 and 11 kr.

The comparison between soaked and dry series revealed some interesting facts. Considering the soaked series alone it was found that the maximum number of flowers obtained in 12 kr-soaked irradiated lot with a mean flower production of 58, closely followed by 15 kr. with an average of 57 flowers. The minimum flower production was noted in 5 and 7 kr.

But in the dry irradiated series the average flower production in both 13 and 15 kr was low, the average being 48 and 49 respectively. In the dry series the maximum flower was noted in 9 kr with an average of 53 flowers.

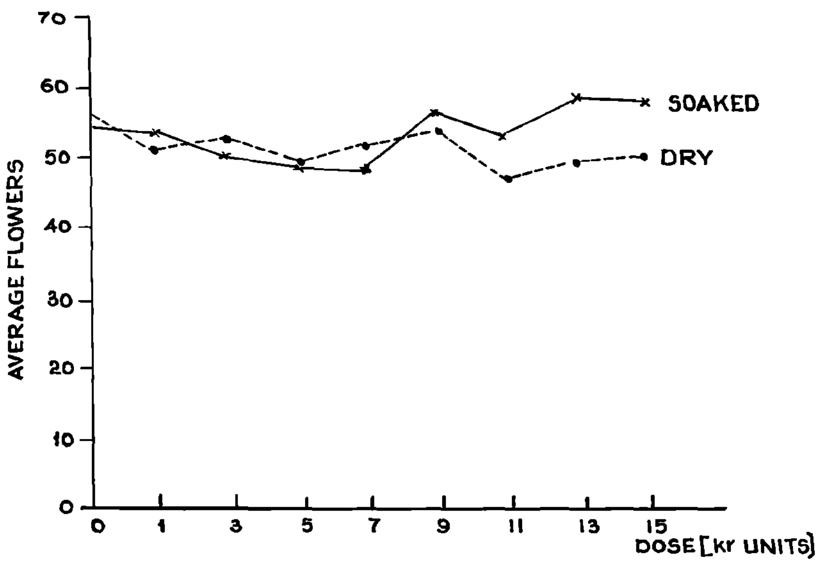
It was also found that in the soaked series, the control produced an average of 54 flowers which was lower than those in 9 , 13 and 15 kr-soaked treatments.

Regression analysis for the average flower production was also conducted . Both linear and quadratic models fitted to the data were found to be not significant when tested. This showed that there was no relationship between X-ray dose and flower production.

Fig. 8. Graph showing the average flower production.

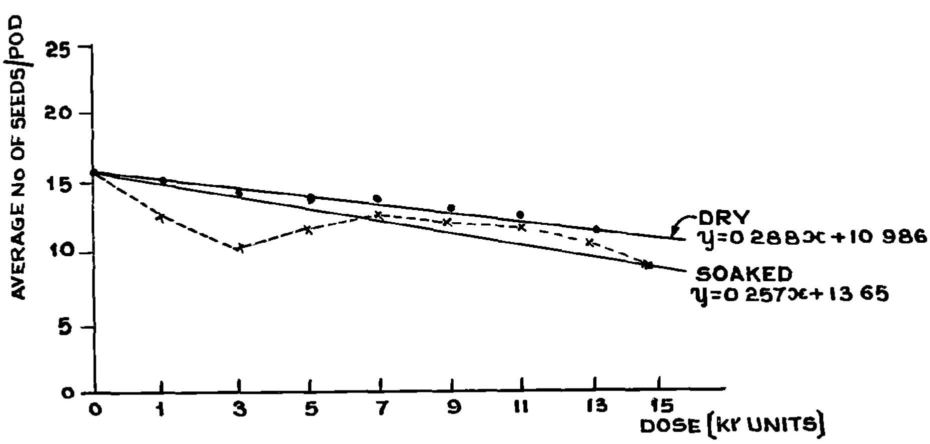
Fig. 9. Regression lines and regression equations for the average number of seeds per pod.

FIG: 8



AVERAGE FLOWER PRODUCTION

FIG. 9



AVERAGE NUMBER OF SEEDS PER POD

PERCENTAGE OF FRUIT SET

The percentage of fruit set was obtained from the total number of flowers produced and the total number of pods obtained. The data obtained is presented in Table No. II.

From the data it was clear that there was no relationship between dose and fruit set. In the dry series the fruit set in the control was 54.57% , in 3 kr, 55.77% and in 15 kr. 53.54%. The lowest percentage of fruit set was seen in seven kr(45.06%). The regression analysis showed that the percentage of fruit set was not dependent on the dose . Both linear and quadratic models turned out to be not significant when tested.

In the soaked series also there was no significant reduction in the percentage of fruit set as the dose increased. The highest percentage of fruit set was found in control plants (53.8%) followed by 7 and 11 kr (53.6 and 53% respectively). The lowest percentage fruit set was found in 15 kr irradiated lot (46.4%) followed by 1 kr (46.8%). In general there was a reduction in fruit set in lower and higher doses than in the medium doses. The regression analysis was carried out Both linear and quadratic models fitted to the data were found to be not significant. The F-ratio was found to be only 0.51. The results indicated that the fruit set was independent of the dose.

AVERAGE NUMBER OF SEEDS PER POD.

The regression analysis (table No. XVIII) showed that in the dry series there was an inverse relationship between dose and seed set. A linear model was fitted to the data of dry series ($y = 0.288x + 10.906$). The F-ratio calculated was 166.58 which was highly significant. The calculated $r^2 = 0.97$ indicate that the linear model was a good fit to the data. Thus the percentage of seed set was linearly related to the dose ie the seed set decreased as the dose increased.

The regression analysis of soaked series is given in Table No. XVII. A linear model was fitted ($y = 0.257x + 13.65$) and when tested it was found that the F-ratio was 7.88 which was significant at 5% level. The r^2 calculated was found to be 0.53 which indicated that the linear model was just satisfactory, from fig.8 it could be seen that there was a depression in average number of seeds in 1 kr and 3 kr, both of which deviated from linearity. In 5 kr and 7 kr there was further increase in the seed set. From 7 kr to 11 kr the average seed number per pod was almost constant. From 11 kr onwards the seed number was again reduced. Thus the seed set was found to

T A B L E XVI

Average number of seeds/pod, 1000 seed weight and % of abnormal anaphases.

Dose in kr units	Average number of seeds/pod		1000 seed weight		% of abnormal anaphases	
	Dry	Soaked	Dry	Soaked	Dry	Soaked
Control	15.9	15.6	71.05	70.33	0	0
1	14.8	12.8	70.54	68.39	2.8	6.4
3	14.2	10.5	68.93	69.86	4.5	6.7
5	13.8	11.5	69.52	69.01	4.9	5.6
7	13.6	12.6	70.37	70.52	6.7	7.4
9	12.9	12.1	68.97	71.38	8.8	13.2
11	12.7	11.9	68.05	69.27	9.3	15.9
13	11.4	10.5	66.28	66.79	10.3	17.5
15	10.8	8.9	65.44	64.82	12.4	18.0

TABLE XVII

Analysis of variance for testing the significance of regression - Average number of seeds per pod (soaked)

Linear model ($y = 0.257 + 13.65$)

Source	Sum of squares	D f	Variance	F-ratio
Total	28.056	8		
Due to Regression	14.8612	1	14.8612	7.88**
Deviation from regression	13.1948	7	1.8849	

* Significant at 5% level $r^2 = 0.53$

T A B L E XVIII

Analysis of variance for testing the significance of regression, average number of seeds per pod (dry series)

Linear model ($y = 0.288x + 10.986$)

Source	Sum of squares	Df	Variance	F-ratio
Total	20.806	8		
Due to regression	20.231	1	20.231	246.72**
Deviation from regression	20.575	7	0.082	

** Significant at 0.1% level $r^2 = 0.97$

be less in low and high dosages, and in medium doses the seed number per pod was found to be relatively higher.

A comparison between the two curves revealed that there was significant difference between the soaked and dry seeds in all doses. This difference seemed to be maximum at 3 kr. At medium doses there appears to be little difference between dry and soaked series.

WEIGHT OF 1,000 SEEDS

The weight of thousand seeds determined from a random sample is given in table No. XVI.

The linear model was found to be not a satisfactory fit to the data so that a quadratic model was fitted. When tested it was found that the F-ratio was not significant in both series. The low F-ratio showed that the seed weight was not dependent on the dose. In the soaked series the maximum weight was found in the 9 kr treated seeds. In the dry series the maximum weight was obtained in the control and the minimum in the 15 kr. Much inconsistancy was seen in between doses. But the differences were not statistically significant.

MISCELLANEOUS OBSERVATIONS.

Pod characters.

Little variation was noted in X_1 regarding the fruit characters. One plant was observed in 13 kr dry irradiated lot with black coloured pods. In normal plants the colour of the pods was straw white when dried.

One plant in the 13 kr soaked was found to have uniformly larger pods and larger seeds with greater number of seeds than the control plants and all other plants in the irradiated lot.

Seed characters.

Three seed colour changes were observed in X_1. One plant, found in 3 kr - dry irradiated seeds, produced straw white, normal sized pods but with buff coloured seed instead of the normal red. The size of the seeds was larger than the normal. This plant gave only 5 pods and a total of 40 seeds.

Two plants, one each in 7 kr. dry irradiated and 13 kr -soaked irradiated produced greyish coloured seeds. The seeds of the plant in 7 kr dry were found to be larger than the normal, while the other plant produced normal sized seeds. Both of them gave fewer number of pods and seeds. The former plant gave 13 pods and a total of

128 seeds and the latter one gave 9 pods and a total of 82 seeds.

Two plants, one each in 11 kr soaked irradiated and 13 kr dry irradiated gave seeds with brownish mottlings. There was no size difference between these and the control.

Thus a totalof five plants were obtained with seed colour changes. These were carried on to the 2nd generation for further study.

Abnormal sterile mutants. (Plates 2 and 3)

Two plants - one in 1 kr dry irradiated and the other in 7 kr soaked irradiated.- were found to be abnormal in growth . The leaves were crinkled, without any well developed branches. These were sterile . One plant failed to flower and the other flowered but failed to set fruits.

CYTOLOGICAL EFFECTS OF X-RAYS

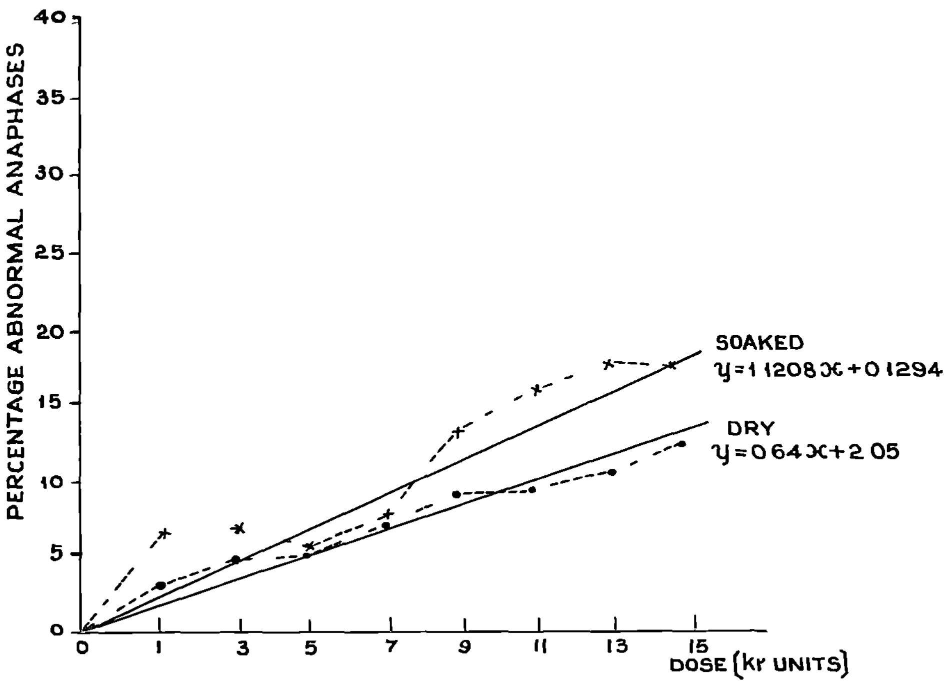
Frequency of abnormal anaphases.

The percentage of abnormal anaphases is given in Table No. XVI.

For getting an idea on the dependence of abnormal anaphases on dose, the regression analysis was carried out . A linear model was fitted to the data of dry series.

Fig. 10. Regression lines and regression equations for the percentage of abnormal anaphases.

FIG 10



PERCENTAGE OF ABNORMAL ANAPHASES

TABLE XIX

Analysis of varianfe for testing the significance of regression. % of abnormal anaphases.

(Dry series) Linear model $y = 0.64x + 2.05$

Source	Sum of squares	Df.	Variance	F-ratio
Total	125.289	8		
Due to regression	105.272	1	105.272	36.89**
Deviation from regression	20.017	7	2.859	

** Significant at 1% level $r^2 = 0.84$

T A B L E 20

Analysis of variance for testing the significance of regression of % of abnormal anaphases (soaked series)

Linear model ($y = 1.1208\ x + 0.1294$)

Source	Sum of squares	D f	Variance v	F-ratio
Total	307.02	8		
Due to regression	285.0165	1	285.0165	90.67**
Deviation from regression	22.0035	7	3.14335	

** Significant at 1% level

$$r^2 = 0.92$$

The model fitted was ($y = 0.64x + 2.05$). When tested this model was found to be a good fit. (Table XIX) . The F-ratio was significant at 0.1% level and the r^2 calculated was 0.84. These high values indicated that the linear model was a good fit for the data. There was an increase in the frequency of abnormal anaphases as the dose increased.

The linear model fitted to the data of soaked series ($y = 1.1208x + 0.1294$) was also found to be highly significant, suggesting an exact linear relationship. (Table XX). The r^2 calculated was 0.92 indicating that the linear model was a good fit. Here also there was a linear increase in the frequency of abnormal anaphases as the dose increased.

The regression lines for the soaked and dry series are represented in fig. 11.

An examination of the data revealed that in all doses, the frequency of abnormal anaphases is higher in the soaked series than in the dry series. This indicates that the soaked seeds are more sentitive to X-rays than the dormant seeds.

<u>Types of chromosomal aberrations.</u>

The main observable chomosomal effects of X-rays were the anaphase bridges and fragments. The anaphase bridges appeared in singles, doubles and in multiples. In many cells, bridges appeared in pairs. Plates 4 to 10 shows the types of choromosomal aberrations observed in the root tips of irradiated seeds.

Apart from the anaphase bridges and fragments some other abnormalities were also noted. Among this clumping of chromosomes at metaphase was important. Clumping was especially common in root tips obtained from seeds irradiated with high dosages. Lagging of chromosomes and precocious separation of chromosomes were also observed. Micronucleus formation was noted in some cells.

OBSERVATIONS ON SECOND GENERATION (X_2)

The role of ionizing radiations is to induce changes in the chromosomes and genes. The X_1 plants raised from the X-radiated seeds will often heterozygous for the various genetic changes induced by the radiation. After self fertilization they give rise to an X_2 generation. In the X_2 and in the later generations (x_3 or x_4) the mutants segregate out. So an analysis of the X_2 generation is very essential to understand the types and extent of mutations induced by the X-irradiation.

In the present investigation also an X_2 population was raised and kept under observation. The seeds were grown in the field and the mutant plants were recorded.

Observations were confined to morphological characters of the plants only. A large control population was also maintained for comparison.

Germination in X_2

Slight fluctuations were noted in the germination percentage of the seeds belonging to the various doses. Table No.21 gives the germination percentage observed in each treatment.

The seeds of the five plants, which gave seed colour changes in X_1 were sown separately. In these groups the germination percentage were found to be greatly reduced as shown in table No.22. This reduction in germination percentage was especially evident in plant Number 1, 4 and 5.

Seedling abnormalities.

Chlorophyll changes.

From the X_2 population a total of nine albino seedlings and seven xantha seedlings were noted. All of them were lethal and died within a few days.

A large number of seedlings with chlorophyll spotting on the first leaves was observed. The spotting varied in intensity and extent.

Crinkled and distorted plants.

Many crinkled and distorted seedlings were noted.

TABLE XXI

Germination Percentage in X_2

Dose (kr Units)	Dry	Soaked
Control	98.8	98.6
1	96	90.4
3	98.4	91.2
5	96.8	90.4
7	97.6	92
9	97.6	89.6
11	96.8	96
13	96.8	94
15	89.6	96.8

T A B L E XXII

Germination % observed in Seed colour change plant progenies.

Plant No.	No. of seeds sowed	No. of seeds germinated	% of germination
I (isolated from 3 kr dry)	40	6	15
2 (isolated from 7 kr dry)	135	120	88.9
3 (isolated from 11 kr soaked)	130	105	80.7
4 (isolated from 13 kr soaked)	75	34	45.3
5 (isolated from 13 kr dry)	130	18	13.8

T A B L E XXIII

Frequency of abnormal seedlings in X_2

Dose (kr units)	Dry	Soaked
Control	-	-
1	7	15
3	5	13
5	9	11
7	12	15
9	14	17
11	11	13
13	19	21
15	17	25

These appeared extremely abnormal. The growth of these was found to be suppressed in many cases. Some produced normal leaves later.

Many plants which look perfectly normal at the time of germination remained stunted without proper vegetative growth. Many of these plants failed to flower.

The total frequency of abnormal seedlings recorded is presented in Table No. XXIII.

Among the X_2 of the seed colour mutants the percentage of abnormal seedlings was high.

MORPHOLOGICAL MUTATIONS

Growth character.

It is recalled here that the normal plants of the variety "African" is an erect growing type without any spreading or twining habit. The branches grow in an upward direction, so that the whole plant grows in a very compact manner. This is a constant character of the variety.

Many variations to the above character were noted.among the irradiated progeny. Some of the more conspicuous types were the following:

i. Twining types.

These types exhibit the twining tendency even in the seedling stage. Five plants were identified with this habit.

ii. Profusely branching and spreading types.

Two plants were extensively branching and spreading in habit.

iii. Dwarf mutants.

These were very prominent and easily identifiable due to their very characteristic appearance. The branches were fewer and grew almost parallel to the surface and the branches arose from the two sides of the stem. Four plants were noted in this type.

iv. Straight stemmed mutants.

One plant was noted to be of this kind. The stem was straight, without any prominent branches . The leaves were crinkled and the plants appeared to be abnormal which failed to flower.

Plates 10 - 17 represents the various morphological mutations observed in the X_2 population.

Flower characters.

Flower colour changes were of common occurrance in the population. The normal flower possessed a light purple 'standard' and wings with violet colouration. The most notable changes were to pure white and to yellow. Among this group three categories were observed.

1. Flowers pure white
2. Flowers pure yellow
3. Standard yellow and 'wings' white.

Apart from the above changes many other less notable changes were also observed. Some of which are the following:

1. Standard pink with wings white
2. Standard bluish violet with the same colour on the wings.
3. Standard bluish violet with wings light pink.
4. Standard bluish with wings white.

Apart from the colour changes other abnormalities were also noted. In one case it was found that the flowers were deformed and failed to open. When examined this was found to be due to the fusion of the floral parts.

Pod characters.

Many variations found to occur in the X_2 affecting the pod characters. These mainly affected the size, and colour of the pods. The various types of pods obtained are represented in plates 18 - 21.

The colour of the pods of the normal plants was straw and the pods were small. Colour variations included blackish, brownish, reddish white, reddish white with deep purple streaks on the pods and light greenish. The size of the pods obtained from various mutant plants varied from large to normal sized to small. A few plants gave very large pods with larger seeds.

SEED COLOUR MUTATION.

Changes in seed colour was one of the most interesting things observed during the course of the experiment. It was earlier stated that five plants were obtained in the X_1 generation with colour variations. These five plants segregated in X_2 for various seed colours. The following are the various colour mutations isolated from the five groups.

Plant No.1.

This plant was obtained from 3 kr - dry irradiated lot. It gavebuff coloured seeds in X_1. In the X_2 five plants reached maturity. Out of this five, two gave buff coloured seeds, 1 red coloured seeds as in normal plants, one gave seeds with brown mottling and one produced red coloured seeds with black mottlings. One of the buff seeded plants was of the spreading type. The brown mottled plant gave pods with a blackish colour when ripen.

Plant No.2

This plant in X_1 produced greyish mottled seeds. In this class 73 plants flowered and set seeds. The following types were obtained.

Seed colour	No. of plants.
Greyish mottled	22
Brown mottled	8
White with Brown eyes	7
White with deep red eyes	6
White	4
Yellowish white	3
Light red	5
Red (normal)	18

It was noted that three of the greyish seeded plants gave larger pods and seeds than the normal plants. The classes white with deep red eyes and light yellow, produced uniformly smaller seeds. In other cases the seeds were of the same size as that of the normal plants.

Plant No.3.

This plant produced brown mottled seeds in the X_1. In this class, 63 plants reached maturity. The following seed types were isolated.

Brown mottled	23
Red normal	27
White with brown hallow around the eye	3
Brown	3
Light red	5
Greyish mottled	2

No changes in colour was noted to be associated with any morphological characters.

Plant No.4.

The original colour of this plant in X_1 was greyish mottled. 18 plants reached maturity. The segregation of the colour mutants was as follows:

Greyish mottled	7
Red	4
Red with black mottlings	2
Brown mottled	3
Very light red	2

Plant No.5.

The colour in the X_1 was brown mottled. 14 plants flowered and set seeds. The following types were recorded.

Brown mottled	3
Red	2
Red with black mottling	3
Brown	1
White with red eyes	1
White with brownish eyes	1
Greyish mottled	3

One of the red seeded with black mottling was dwarfish in nature. One of the brown mottled plants gave blackish pods.

In the X_2 seed colour changes were also observed in the irradiated population raised from the apparently normal X_1 seeds. The following colour changes were noted.

1. The half red-white type. One plant was noted in the 5 kr dry irradiated lot for its twining habit. This plant when matured gave seeds in which half of the seed - the eye portion - is red and the other half white. Only three pods were obtained. Seed set seemed to be very low.

2. Greyish mottled. Three plants were noted which gave larger sized greyish coloured seeds. Two of these were dwarf plants. One plant produced larger pods also.

3. Brown mottled. Five plants were noted with brown mottled seeds . One of these was a dwarf plant.

4. White with black eyes. One plant was noted which gave white seeds with blackish colour around the eye portion.

5. White seeded type. The plants were isolated which produced white seeds. In one case the seeds were a little larger than the normal.

Plates 22 and 23 represents the various seed types obtained in X_2.

OTHER MORPHOLOGICAL MUTATIONS.

Large leaved mutant. (Plate 24)

One plant in the X_2 (5 kr dry) was very prominent due to its vigorous growth, much larger leaves and twining habit. The leaves were thicker than the normal leaves. Flowering was delayed for about 3 weeks . It produced only three pods and the pods contained very few seeds.

Chimaeric plant.

One plant in 3 kr dry irradiated group produced one branch which was a chimaera. The leaves of one side were normal, and in the opposite side the leaves were of the mutant type with green and yellow patches. Plate 25 shows the nature of the chimaera.

Anthocyanin production.

One plant was observed in the 3 kr dry treated group, in which the peduncle was characterized by a deep purple colour. The colour was observed in no other part of the plant.

In two plants - noted in 7 kr-soaked irradiated series, and 13 kr-dry irradiated series,the lower part of the stem possessed a deep purple pigment. This colouration appeared diluted in the upper portions .

Another interesting case of pigmentation was exhibited in one plant in 9 kr-dry irradiated group.

In this plant the nodes of the stem possessed a deep purple pigmentation. The pigment extended about one cm. to the upper and lower parts of the nodal region.

Three plants were noted / two in 13 kr - and one in 15 kr-soaked irradiated - in which the sepals were characterized by purple streaks.

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D I S C U S S I O N

GENERAL CONSIDERATIONS.

In spite of a large amount of work to understand the mechanism of action of X-rays, little progress has been attained till recently. The dominant thought upto 1937 was that metabolic reactions play no part in the primary action of X-rays and that any detected metabolic disturbances are a manifestation of degenerative processes which succeeded the primary damage to cell growth and the nucleus (Scott. 1937). The target theory dominated the field. The classical experiments of Dale (1940) broke the ground for the introduction of the indirect action theory, so repeatedly presented by Fricke (1934). The great advances made in the field of free radical reactions and the production of mutations by those free radicals, found in irradiation of water, have demonstrated the importance of this mode of action of ionizing radiation on the living cells. It has been repeatedly shown by biologists and chemists (Barron 1954; Hart 1958) that when water containing dissolved oxygen is irradiated with ionizing radiation such as X-rays, there is the ultimate formation of hydroxyl radicals and hydrogen atoms: $H_2O \rightleftharpoons HO + H$. In the presence of dissolved oxygen the hydrogen atoms combine to form O_2H radicals. The O_2H radicals may further be reduced by hydrogen atoms to hydrogen peroxide. The hydroxyl radicals may also give hydrogen peroxide. These reactions are conditioned by many factors such as the oxygen concentration, rate of irradiation, temperature etc. All these radicals formed are oxidising agents. It was predicted (Barron 1954) that ionizing radiations would oxidise the oxidation - reduction systems of biological importance.

EFFECT OF PRE-SOAKING ON X-RAY SENSITIVITY.

It has been shown by many workers that pre-soaking enhances the radio-sensitivity of the seeds. (Moore and Haskins 1933., Gustafsson 1940, 1947., Wertz 1940., Gelin 1941., Mackey 1951., Shastry and Ramiah 1961). The same conclusion can also be deduced from the present investigations.

It was found that germination percentage was unaffected by the X-ray treatment in the dry series, but reduced significantly in the soaked series. This is in line with the evidences presented by Gustafsson (1947), Lefort (1954) and Lesley and Lesley (1957).

Lethality was higher in the soaked seeds than in the dry seeds in all the doses. In both cases, a linear relationship between dose and survival was found to hold good. Gustafsson (1947) and Stapleton and Hollaender (1952) after their careful studies arrived at similar conclusions.

It was observed that at all doses, the frequency of chlorophyll abnormalities was higher in the soaked than in the dry seeds. In both cases there were a linear increase in the frequency of chlorophyll abnormalities, though this relationship was more evident in the soaked series.

Caldecott (1956) and Lesley and Lesley (1957) reported greater reduction in seedling height in the soaked seeds than in the dry seeds. The present studies confirmed the findings of the above authors. It was found that in the dry seeds there was no significant reduction in seedling height. But in the soaked seeds there was a significant decreases in seedling height as the dose increased. The results showed that the soaked seeds were injured more severely than the dry ones by the X-ray treatment.

Increased XI sterility was noticed in the soaked seeds than in the dry seeds in almost all doses employed except at the highest dose. At the highest dose there was no significant difference between the two series. Comparable results came from Gustafsson (1947) and D'Amato and Gustafsson (1948) in barley.

Gelin (1941) found that the percentage of abnormal cell division increased with the increase in water content. In the present investigation it was found that the percentage of abnormal anaphases was much higher in the soaked seeds than in the dry seeds at all doses studied.

Thus all the observations tend to prove that soaking of seeds in water prior to irradiation enhances the X-ray sensitivity of the seeds. Ehrenberg (1953) observed that at comparatively low dosages, the pre-soaked seeds reached higher levels of mutability than the dry seeds. Thus he showed that with a dose of 625 r in barley, the mutation rate in the soaked seeds was seven times higher than that of dormant seeds.

The actual reason behind the increased sensitivity of pre-soaked seeds is not clear. The increase in volume of the seed can be one of the reasons for the increased sensitivity. Such a conclusion was first advocated by MacKey (1951). The increased volume provided larger area for the action of radiation. Caldecott (1954) stated that the increased sensitivity was probably the result of the production of chemically active radicals from water. This was in line with the original findings of Fricke (1934, 1935). Fricke and Demerec (1937) found that 2 Å X-rays was as efficient as ordinary X-rays in producing mutations. This apparent violation of the "target hypothesis" was explained by them to be due to the production of mutation via the intermediation of "activated water".

EFFECT OF X-RAYS ON GERMINATION.

In the present investigation it was found that the germination percentage was not dependent on dose in the dry series. But in the soaked series there was a linear relationship between dose and the germination percentage.

Many workers (Micke 1958., Bora and Rao 1959., Gottschalk and Scheibe 1960., Shastry and Ramiah 1961., Jan Sjodin 1962 and Katayama 1963) reported that in the dormant seeds the germination ability was unaffected by the X-ray treatment. The present study confirmed the above reports.

In the soaked series germination percentage was found unaffected by low and medium doses (1000 to 9000 r), but at higher dosages (11000 to 15000 r) it was reduced significantly. However, further investigation with still higher doses than those used in the present study is required to establish the linear relationship between higher doses and germination percentage.

It is known that germination of the seeds does not follow cell division. Smear preparations of the tips of radicles when it was ½-1 cm. in length failed to reveal any dividing cells.

Since the main effect of X-rays is on the chromosomes, the deleterious effects will be manifested only when the cells begin to divide. Those cells which are too much affected by X-rays will be damaged due to abnormal mitosis that follows. The genetic complement of a cell in which a large number of chromosome breaks have been induced will remain intact as long as the cell does not divide. Since the germination of the seeds does not follow the cell division, it can be concluded that the germination percentage is unaffected by the X-ray dosages.

Confirmatory evidence to the above conclusion had been reported by Schwartz and Bay (1956). They noted normal germination around 5,00,000 r units. The dose caused total inhibition of mitosis as evidenced from the fact that the root tip smears showed no dividing cells and the growth ceased completely after about five days. The germination and the early growth, they suggested, were due to the elongation of the cells already present in the embryo at the time of irradiation.

But the problem ramains why there was a significant reduction in germination percentage at high doses in the soaked seeds. It was noted earlier that the radio-sensitivity is dependent on the physiological condition of the seeds (Gustafsson 1944). Soaking of seeds initiates a series of changes within the embryo. Scott (1938) had early shown that at high doses of X-rays the enzymatic activities were inhibited. Such inhibition of enzymatic and hormonal activities may cause a breakdown of the cellular metabolism. Again, active oxygen containing radicals such as H_2O_2 and O_2H produced under the impact of irradiation may oxidise the biological systems at work in the embryo(Barron 1954). This may again inhibit the germination of seeds.

Barron and Bonzell (1950) and Barron (1954) reported that the enzymatic changes induced by 10,000 r of X-rays are reversible which is especially true in the case of the cytochrome - oxidation - reduction system. Still higher dosages may cause permanent alteration in the enzymatic systems which may manifest in the germination following irradiation. This might be yet another reason for the reduction in germination percentage at higher dosages in the soaked series.

EFFECT OF X-RAYS ON SURVIVAL.

In the present investigation it was found that the survival percentage was linearly related to the dose, ie. survival decreased proportionately as the dose increased.

Linear relationship between dose and survival percentage was also reported by Wohrmann (1955)., Giles (1956)., Ehrenberg (1955) and Sjodin (1961). Yagayu and Morris (1957) observed that in dormant tomato seeds the survival percentage was not reduced by doses upto 10,000 r and above that there was the expected linear reduction.

Even though the linear model fitted to the survival percentage of dry series was found to be highly significant, variations from linearity was noted in 1 kr treated seeds. In the dry seeds, 1 kr caused no reduction in survival. The survival curve in the dry series is almost sigmoidal in nature. The initial flat portion of the sigmoid curve indicated that no macroscopic biological effect was detected until a certain amount of latent action of radiation has accumulated (Fano 1954). This rule was established in the radiobiological literature through the works of the exponents of the "target theory". Drawing upon the above rule it can be said that 1 kr is not strong enough to cause an accumulation of effects ("hits") to produce a visible effect on the organism.

But the typical sigmoid curve differed from the one which was obtained in the present investigation. In a typical sigmoid curve, the flattening is seen in both low and high doses. This flattening at high doses means that the effect is reaching completion (Fano 1954). But in the curve obtained in the present investigation, there was no such flattening at high doses. This was clearly due to the fact that the doses employed were not high enough to cause a saturation effect. The lowest percentage of survival noted was only 62% at 15 kr. The LD_{50} was not reached for which still higher doses were required.

Slight deviations from linearity was also observed in the case of soaked seeds. The deviation was seen in 1 kr which gave only 83.8% survival compared to the 100% in the dry series. This finding is in line with the observation of Ehrenberg (1953) who found that in the soaked seeds, comparatively lower doses were very effective in inducing mutations.

GROWTH OF THE SEEDLINGS.

Growth of plants is a complex character which is quantitative in nature. Growth is also dependent on many environmental factors. The effect of X-rays on such a complex character is more difficult to evaluate.

The present investigations indicated that the seedling height in the dry series was not related to the dose. But in the case of soaked seeds, height was significantly reduced at higher dosages used.

The effect of X-rays on growth is a controversial subject. Many authors reported an acceleration of growth following X-irradiation of seeds (Kornicke 1904., Miege and Coupe 1914, Goodspeed and Olson 1928). No such acceleration was noticed in the present study in any dose.

The reduction in growth in the soaked series is in line with the evidences presented by other workers (Capizzaro 1926., Shastry and Ramiah 1961., Scossiroli et al 1961 and Micke 1961). But the reduction in height was not linear as reported by Beard et al (1958). It was found that the height of plants and dose related curvilinearly.

The absence of any effect on seedling height in the dry irradiated seeds might be due to the fact that the doses employed were not high enough to cause any suppression of growth. Absence of any visible effect on height after X-irradiation of dry seeds was previously reported by Kuzin (1955).

Even in the soaked seeds, it was found that the reduction in heightwas exhibited only in the seedling stage. The height measurements at the 7th week failed to show any significant difference between the various treatments.

Growth takes place by repeated cell divisions. The injurious effect of radiation on chromosomes is manifested in the disturbed divisions. If many cell primordia in the embryo were affected by radiation, then such cells could not complete the division cycles successfully which result in a cessation of growth in the early seedling stage.

Recent studies by various workers (D'Amato 1961., Bhaskaran and Swaminathan 1961., Swaminathan 1961., Shastry and Ramiah 1961) indicated a strong intrasomatic selection going on in the treated plants. This intrasomatic selection eliminates the damaged and abnormal cells leaving the normal cells to proliferate. Thus, further growth of these seedlings occurs by division of normal cells. So normal growth occurs in the later stages of the growth. Evidently such a phenomenon is responsible for the absence of any significant difference in height in mature plants. This was further evidenced from the weekly height measurements recorded. So the reduction in growth exhibited by the seedlings was transient.

CYTOLOGICAL EFFECTS OF X-RAY TREATMENT.

In the present investigation it was found that the frequency of abnormal anaphases gave a linear relationship with the dose. In all doses employed the frequency of abnormal anaphases was greater in the soaked seed series than in the dry seeds. The main observable anaphasic abnormalities were the bridges and fragments.

From his elaborate experiments on Tradescantia, Giles (1943) had shown that the main chromosomal aberrations induced at the resting stage were chromosomal terminal deletions, chromosome exchanges and chromosome interstitial deletions. So the bridges observed in the anaphase might have been produced by chromosome exchanges.

Chromosome exchanges are two-hit aberrations requiring two independent breaks. The frequency of chromosomal aberrations which involves two chromosomes or two loci in the same chromosome has been observed by Sax and Brumfield (1943) to increase as the square of the X-ray dosages, but those resulting from single break as simple delegations, are directly proportional to X-ray dosage. However, such relationship has been demonstrated to depend on the radiation-time - intensity factor (Sax 1941 and Marinelli 1942).

The linear relationship obtained in the present investigation thus deviates from the above rule. Linear relationship between the frequency of two-hit chromosomal aberrations and X-ray dosages has been reported by Caldecott (1954), Yagayu and Morris (1954) and Basu (1962).

Sax and Brumfield (1943) hypothesized that in two-hit aberrations the breaks are induced by the passage of one electron. This hypothesis has since been shown to be true by Caldecott (1954), and Yagayu and Morris (1957). In such cases the interchanges and dose varied linearly. This might be the reason for obtaining the linear relationship in the present study. Basu (1962) also gave similar explanation for his results.

STERILITY IN X_1

In the present investigation it was found that the sterility of pollen is highly correlated to the dose of X-rays. In both dry and soaked series, there was a linear increase in percentage of pollen sterility, though this relationship was more evident in the dry series. Similar results were obtained by many other workers with different plants(Gustafsson 1944., Freiselben and Lein 1943, MacKey 1951 and Wohrmann 1955).

Contradictory results were obtained by Genter and Brown (1941), Hackbarth (1955) and Zacharias (1956). These authors reported that X-ray treatment caused no significant reduction in pollen fertility.

Chaudhuri and Das (1956) obtained linear increase in pollen sterility upto 40,000 r above which there was a decrease in sterility. They suggested that in some cases the physiochemical changes in the irradiated embryo may practically counteract the effects of higher dosages.

The present results obtained are not compatable with the above reports. In all the doses investigated the pollen sterility was found to increase as the dose was increased. This is in line with the evidences presented by Sjodin (1961). Linear increase of sterility was also reported by Ehrenberg (1955) in barley, Beard *et al* (1959) in barly, Bakendam (1961) in rice and kundu *et al* (1961) in *Chorchorus*.

In the present investigation ovule sterility was also found to be reduced significantly. This is indicated by the average number of seeds per pod. Average number of seeds per pod gave a linear relationship with the dose. Seed setting was reduced as the dose increased.

MUTATIONS IN X_2

Seed types.

Variation in seed coat colour is the most interesting character change observed during the course of the experiment. Such interesting seed coat colour mutations has been reported earlier by Stadler (1931) in *Zea mays* and Sjodin (1961) in *Vicia faba*.

The genetics of the seed coat colour changes is rather complicated. In general the seed coat colour changes can be divided into three:

(i) 'whole colour' changes in which the colour is spread uniformly over the whole seed.

(ii) 'eye-colour' changes in which the change in colour is noted only in the eye portion.

(iii) mottlings in which the colour is present only in certain areas like patches or dots.

The whole colour changes obtained included white, light red, brown and yellowish white. The eye colour changes noticed are, white seeds with brown eyes, white seeds with red eyes, white seeds with bluish mottlings around the eye and white seeds with black eyes. Mottlings included greyish, brownish, blackish and violet.

Since a large number of seed coat colour changes were produced, it should evidently be due to changes in the genetic material. The exact nature of these changes is difficult to interpret without further investigations.

Harland (1920, 1922) proposed four factors as responsible for the 'whole colours' in Vigna. They are 'B', 'N', 'M' and 'R', the various combinations of which gave various colours. According to this scheme the red seeded type is of the genotype 'b n m R.' 'R' is a basic colour gene, in the absence of which the colour will be white. Taking this explanation into account the origin of white seeds can be explained as due to a deletion of the 'R' locus.

Spillman and Sando (1930) proposed eight factors as responsible for seed coat colour in Vigna. They are 'B' (brown), 'R' (red), 'U' (white), 'P' (purple), 'F' (dense bluish mottling), 'T' (sparse mottling) and 'X' (inhibitor gene which inhibits the effect of'F') . The authors stated that various combinations of the eight genes gave various seed coat colours. Deletion or mutation of these factors thus lead to changes in seed coat colour. For example deletion of 'R' locus will lead to the productionof white seeds, and deletion of 'X' produces dense mottlings on the seed coat since the inhibition is removed. The production of other colours is difficult to interpret. If it is assumed that true gene mutation can take place, as shown by Muller (1954) in Drosophila, then it can be interpreted that a mutation of 'b' to 'B' will change the seed coat colour from red to brown, since 'B' is dominant over 'R'. Whether such changes had taken place is doubtful which can be cleared only by further investigations.

THE NATURE OF THE INDUCED MUTATIONS.

The true nature of the induced mutations by ionizing radiation is a debated topic. Muller and coworkers originally considered that X-rays induced true mutations in the gene, and according to them radiation induced mutations were true gene mutations (Muller 1954). But during the past one decade, this concept of gene mutation was severely questioned by various workers. Stadler (1954) stated that most of the induced variations in maize was extragenic in origin. He had questioned the very concept of gene or "true" mutations. He put it "a mutant may meet every test of gene mutation and yet, if it is not capable of reverse mutation there is ground for the suspicion that it may due to gene loss, while, if it is capable of reverse mutation, there is ground for the suspicion that it may due to an expression effect". According to Stadler, the non-lethal variations were probably the result of deletion of the components of a complex locus, leaving at least one of the sub-units intact.

If the above interpretation is valid, then it is only logical to consider that the seed coat colour in Vigna is governed by a complex locus, with many sub-units, the deletion, or alteration of one may effect a change in seed coat colour.

The findings of the modern geneticists is almost on the verge of solving the mutation problem. Benzer (1957) has coined the term "muton" to designate the ultimate unit which when altered produce a change in the organism. Muton is reported by him to be about four to five nucleotide pairs long. Benzer also introduced the term "cistron" as the ultimate unit of function, which is about 4 map units or about 4,000 nucleotide pairs long. Each cistron is a group of pseudoalleles and consisted of a large number of mutons. Any permanent change in a muton will be reflected in the organism as a mutation. The cistron concept in essence is the same as the 'complex locus' of Stadler (1954).

Mc Clintock's (1951, 1955) remarkable analysis on the mutation system in maize revealed certain elements controlling certain characters and functions. Even though there is no definite evidence for the occurrence of such "controlling elements" from outside Zea mays, there are many records of mutable genes and variegated patterns which suggest their existence (Swaminathan 1963). The existence of such elements is not unlikely in Vigna also. The unexpected types of colour changes points to such a possibility.

Separation of complex locus was known to result by irradiation. Thus Davis and Wall (1960) were able to separate the 'V^{by}' locus in Trifolium repens, which resulted in the production of different leaf shapes in the progenies. Confirmatory evidence came from Laughnan (1961) in maize. It is likely that X-ray treatment splitted up a closely linked group of pseudoalleles, the components of which produced various colour patterns in the seed. The presence of modifiers cannot be excluded. This idea is not incompatible with the ideas of Stadler (1954) on the 'complex locus' and Benzer (1957) on the 'cistrons'. A thorough investigation is necessary for the establishment of these ideas.

PRACTICAL UTILIZATION OF THE PRESENT INVESTIGATION

From the many excellent summaries available of the research carried out in different countries on the use of radiations in plant breeding, it is clear that considerable practical achievements have already been accomplished in this field, particularly in Sweden and Germany. Wherever research on mutation breeding was carried out, the experience has been that "induced mutations could increase the yielding capacity of a variety, or on the other hand, left its production intact and improved special qualities of importance in modern agriculture, such as stiffness of straw, earliness, protein content, baking quality, fibre length and grain size". (Gustafsson and Tedin 1954).

The present investigation seems to be of value in the following respects:

1. The different seed colour mutations obtained in the X_2 may be carried forward and selection for high yield and other economic characters may yield new and better varieties.

2. A few plants were obtained in X_2 with larger pods and with greater number of seeds. Selection among the progenies of such plants may give varieties with larger pods and thus with greater yield.

3. A few plants were isolated in X_2 with larger seeds. These may prove to be of value in course of time.

4. From the investigation it was found that the seed colour is the most easily affected character. Further genetic investigation on this character may prove to be of great theoretical significance, because these may give

information on the nature of the genes that controlling the character. Such investigations in maize proved to be of great significance in understanding the natûre of gene and gene action.

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S U M M A R Y

This thesis embodies the results of an investigation carried out to study the effect of X-rays on the common cowpea (*Vigna sinensis*). A pure variety of cowpea (African) was selected, dry and pre-soaked seeds of which were treated with X-rays at the following doses: 1,000, 3,000, 5,000, 7,000, 9,000, 11,000, 13,000 and 15,000 r. units.

Germination, survival of plants, chlorophyll and other morphological abnormalities, growth of plants, pollen sterility, flower production, fruit set, average seeds per pod and 1000 seed weight were studied. The frequency of abnormal anaphases were studied from root tip squashes. Regression analysis was carried out for almost all observations to establish the relationship between doses and the different characters observed.

From the study it was found that dry and soaked seeds differ in their response to X-rays, the soaked ones being more sensitive than the dry ones. Germination percentage was unaffected by X-rays in the dry seeds, but reduced significantly in the soaked seeds. Survival was found to be linearly reduced in both series. Chlorophyll abnormality was higher in the soaked seeds than in the dry seeds. In both cases there was a linear relationship. Seedling height was found to be reduced significantly in

the soaked series, but was unaffected in the dry series. Even in the soaked series, the reduction in height was found to be transient, because later measurements failed to show any reduction in height.

Total flower production was found to be not significantly different between the control and treatments. But among the various treatments, 9 kr produced significantly more flowers than any other doses.

Pollen sterility in both cases showed a linear increase as the dose increased.

Percentage of fruit set was unaffected by the X-ray treatment. The average number of seeds per pod was found to be reduced linearly with the increase in dose. The weight of seeds was unaffected by the treatment.

The frequency of abnormal anaphases was found to increase linearly with the dose. In all doses the soaked seeds gave higher percentage of abnormal anaphases than the dry seeds. Chromosome bridges and fragments were the commonest aberrations noticed.

In the first generation five plants were obtained with seed colour changes. The seeds of these plants were carried to the second generation, where they segregated for various seed colours. About 17 different types of seeds were isolated in the X_2.

From the apparently normal seeds obtained in X_1, 125 seeds from each treatment and controls were selected and an X_2 was raised from these seeds. Various mutations, affecting flower colour, growth habit, pod and seed characters were analysed.

The important results obtained were discussed and possible interpretations of the results were given. The possible value of the investigation was also examined.

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Plate 1. A normal plant of the variety 'African'

PLATE 1

Plates 2 and 3. Two abnormal sterile mutants from the X_1 .

2. from 1 kr dry irradiated

3. from 7 kar soaked irradiated

PLATE 2

nPLATE 3

Plate 4. A normal metaphase with 22 chromosomes.

Plate 5. A normal anaphase.

Plate 6. Cell showing a metaphase plate in which two chromosomes failed to orient in the equaterial plate. A fragment is also visible.

On the left of the plate a cell in telophase showing the organisation of a micronucleus.

Plate 7. An anaphase with one bridge.

Plate 8. Microphotograph of a cell which originally included four chromosome bridges. Two of which were broken up and two can be visible faintly.

PLATE 4

PLATE 5

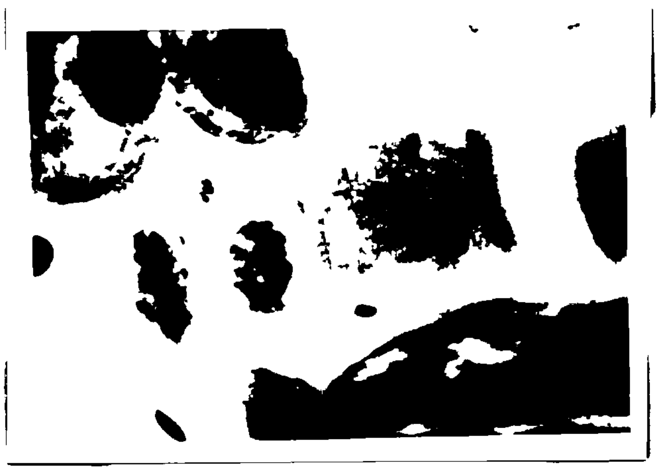

PLATE 6

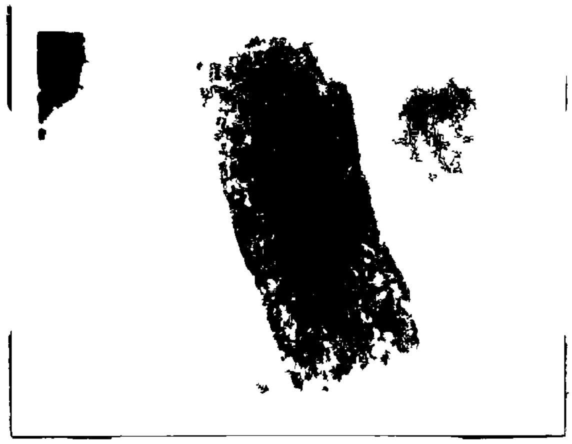

PLATE 7

PLATE 8

Plate 9. The cell in the extreme left shows an anaphase with one fragment. On the right an anaphase showing one bridge with one fragment.

Plate 10. An anaphase with one bridge and two fragments.

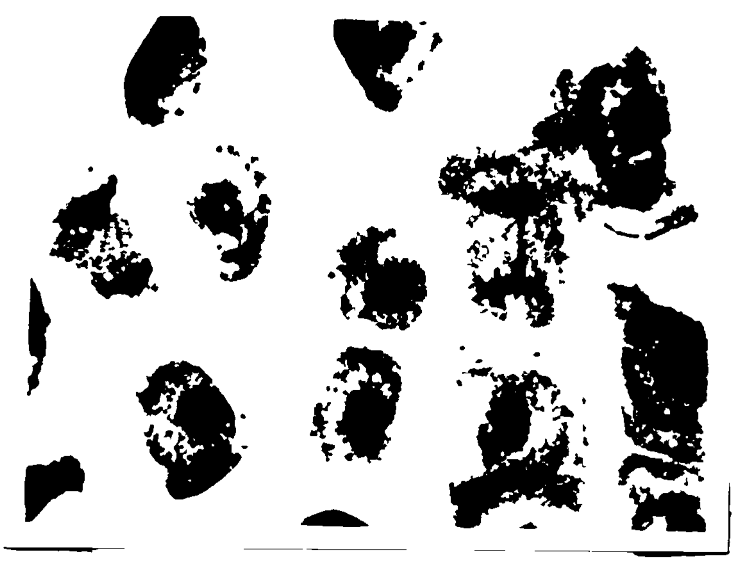

PLATE 9

PLATE 10

Plate 11. A profusely branched and spreading plant isolated from the X_2

Plate 12. A mutant plant from the X_2 exhibiting twining habit.

PLATE 11

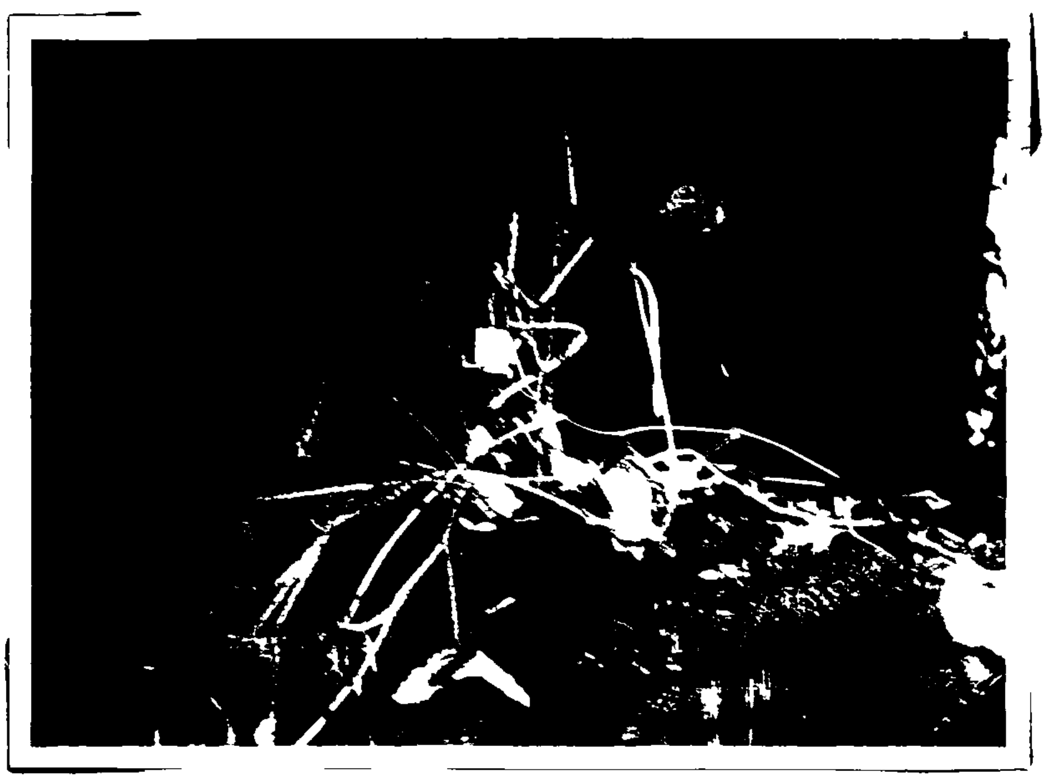

PLATE 12

Plate 13. A mutant plant whose branches and terminal bud exhibits a twining habit. The peduncle was much longer than the normal plants and produced larger pods.

Plate 14 A plant obtained in the X_2 in which the branches exhibit twiny habit. This plant produced much thicker pods.

PLATE 13

PLATE 14

Plate 15. A mutant plant in which some of the branches exhibit twining habit.

Plate 16. A mutant plant whose lower branches exhibiting trailing habit.

PLATE 15

PLATE 16

Plate 17. A dwarf mutant from the X_2 with its very characteristic appearance.

Plate 18. The straight stemmed mutant. The plant is abnormal and sterile.

PLATE 17

PLATE 18

Plate 19. Types of pods obtained in the X_2

1. Large pods with straw white colour.

2. Large and thicker pods with brownish colouration.

3. Large pods with brownish colouration.

4. Medium sized pods - white.

5. Medium sized pods with reddish white colouration.

6. Medium sized pods with light greenish colour.

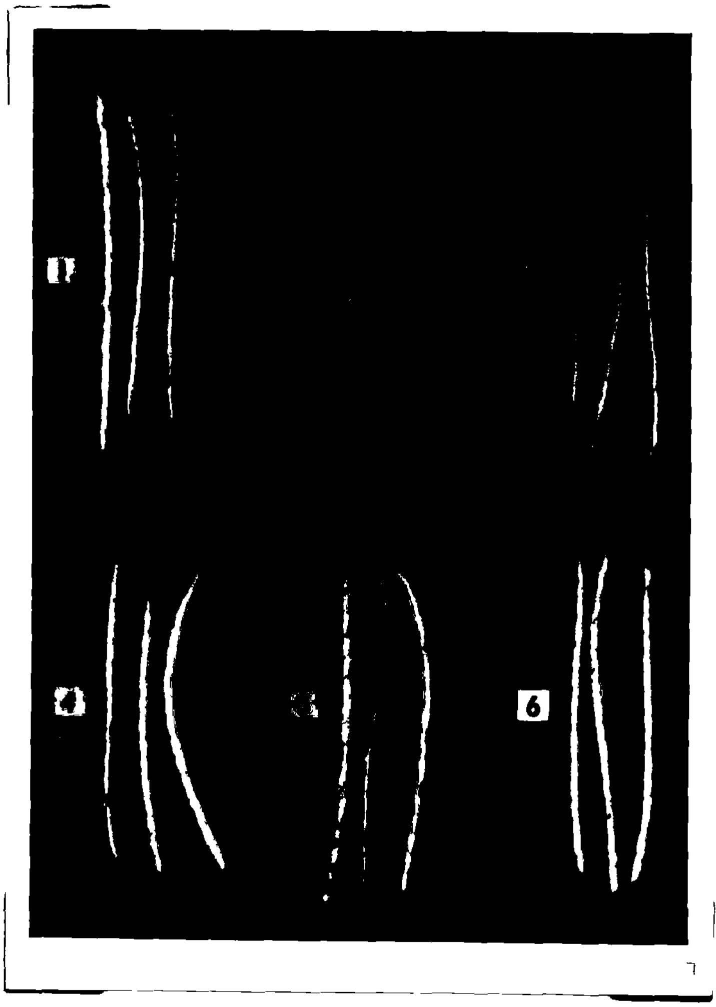

PLATE 19

Plate 20. Types of pods obtained in the X_2

7. Thick flattened, medium sized pods with brown colour.

8. Small pods with reddish white colour and with purple streaks.

9. Small pods with black colouration.

10. Thick flattened pods with straw white colouration.

11. Reddish white medium sized pods.

12. Medium sized thick pods with light yellow colour.

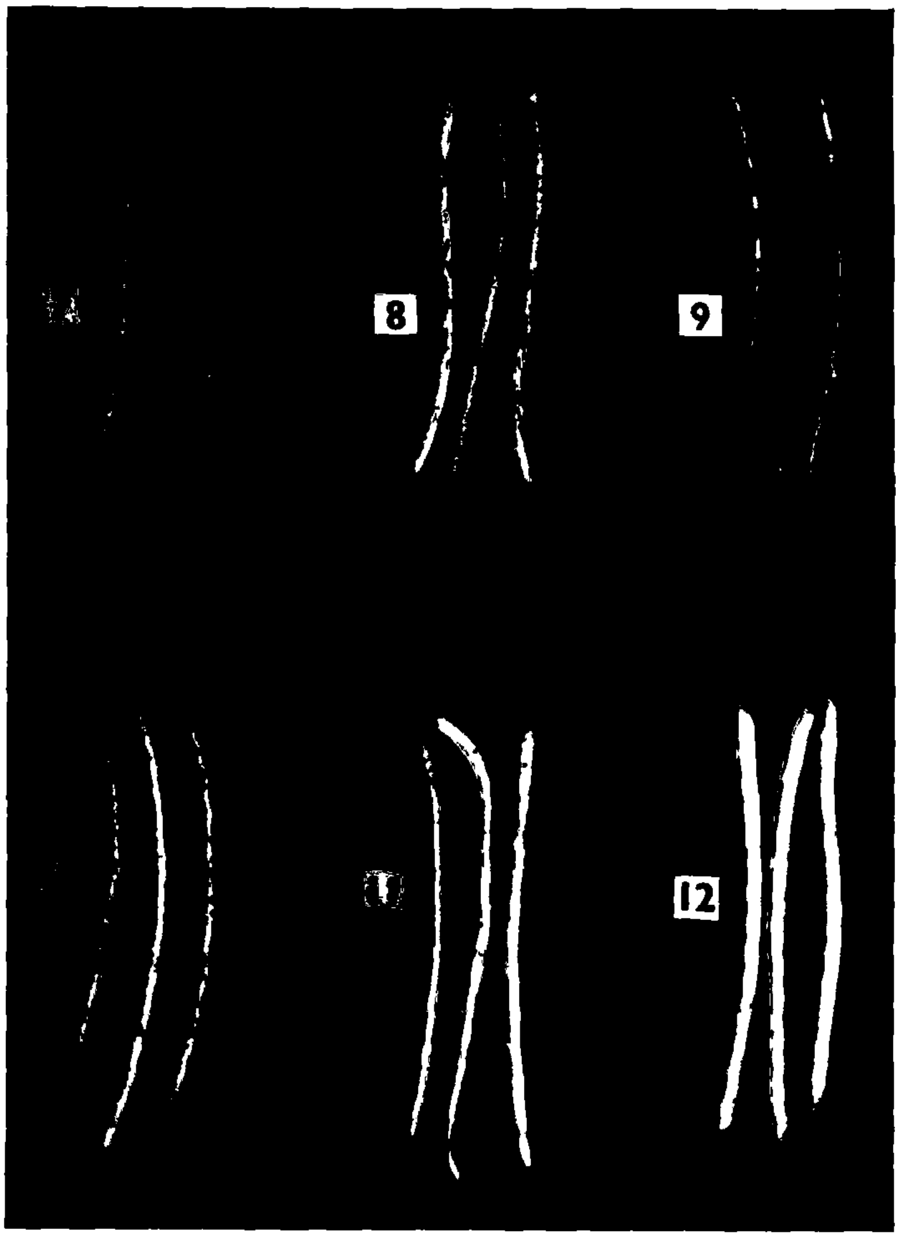

PLATE 20

Plate 21. Types of pods obtained in the X_2

13. Small blackish pods.

14. Small pods with yellowish colouration.

15. Medium sized buff coloured pods.

16. Very small straw white pods.

17. Small pods with light greenish colour.

18; Normal pods of the variety African. - Colour - straw white.

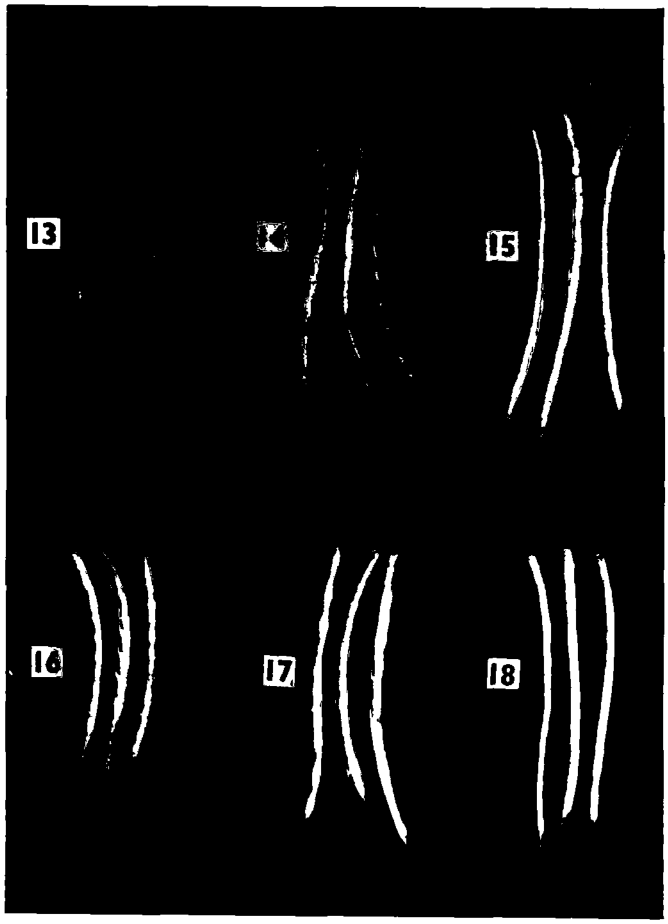

PLATE 21

Plate 22. Types of seeds obtained from the X_2 generation.

1. Normal red seeds of the variety African.
2. Reddish seeds with black mottlings.
3. White seeds.
4. White seeds with brown eyes.
5. White seeds with bluish mottlings around the eye.
6. Greyish mottled large seeds.
7. Densely mottled greyish seeds.
8. Reddish white seeds.
9. Deep brown mottled seeds.

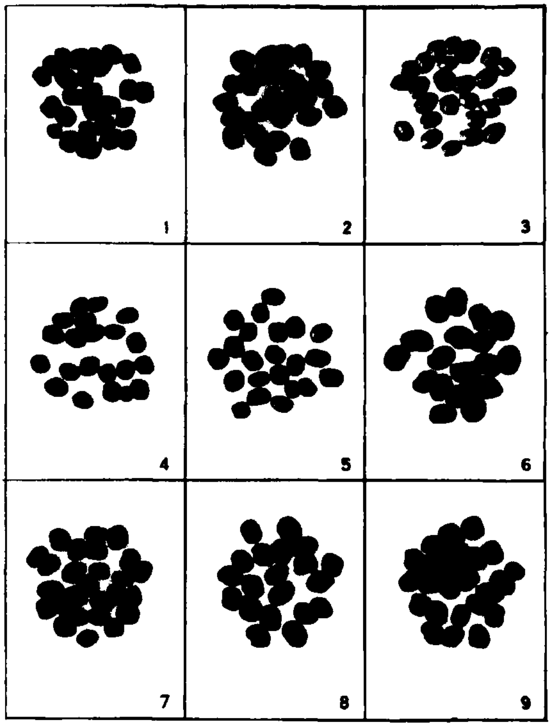

PLATE 22

Plate 23. Seed types isolated from the X_2

10. Half white - half red type.

11. Light yellowish seeds.

12. White seeds with red eyes.

13. Large brown seeds.

14. Small brown seeds.

15. Reddish white seeds with light brown mottling.

16. Deep Ash colour mottled.

17. White seeds with black eyes.

18. Very deep violet mottled.

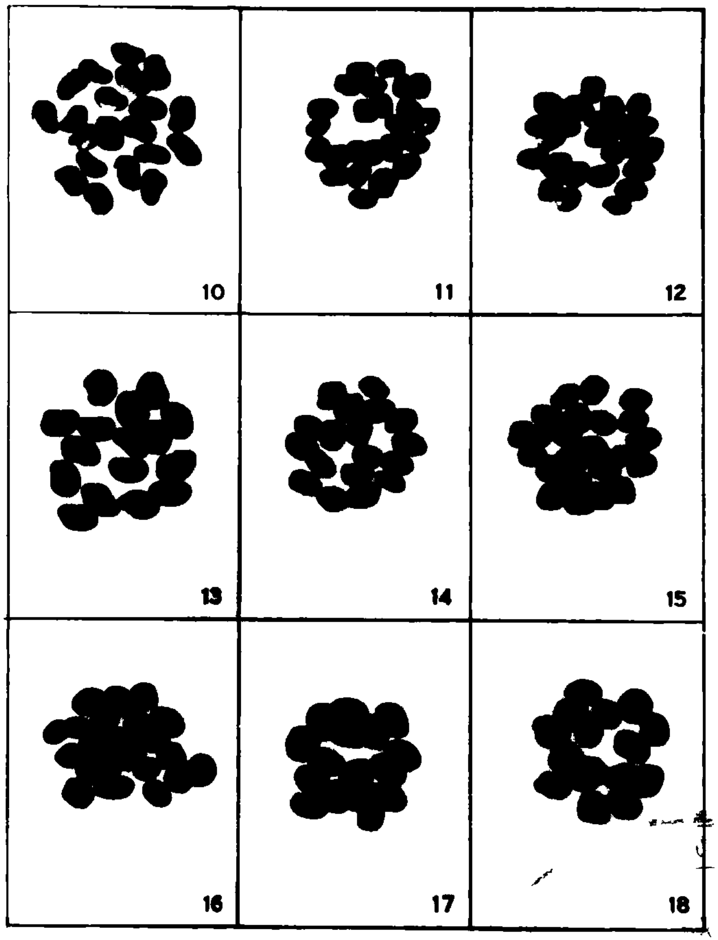

PLATE 23

Plate 24. The large leaved mutant.

Plate 25. The normal and mutated leaves of the chimaeric plant.

PLATE 24

PLATE 25