STUDIES ON THE MORPHOLOGICAL AND CYTOLOGICAL BEHAVIOUR OF X_2 AND X_4 GENERATIONS IN COW PEA (*Vigna sinensis* (L.) Savi)

BY

K. KUMARAN

THESIS

Submitted in partial fulfilment of the requirements

for the Degree of

MASTER OF SCIENCE(AGRICULTURAL BOTANY)

OF THE

UNIVERSITY of KERALA

DIVISION OF AGRICULTURAL BOTANY

AGRICULTURE COLLEGE AND RESEARCH INSTITUTE

VELLAYANI, TRIVANDRUM

1965

CERTIFICATE

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

C.K.N. NAIR
Principal & Addl. Director
of Agriculture (Research).

P. KUMARA PILLAI
Professor of
Agrl. Botany.

Agricultural College &
Research Institute,
Vellayani, Trivandrum,
Date: 31-7-1965.

ACKNOWLEDGEMENTS.

It gives immense pleasure to the author to place on record his deep sense of gratitude and indebtedness to Prof.P.Kumara Pillai, M.Sc., M.S. (U.S.A.), Vice-Principal & Head of the Division of Agricultural Botany and Dr. (Mrs.) Mary K.George, M.Sc., Ph.D.,Junior Professor of Agricultural Botany,Agricultural College and Research Institute,Vellayani for suggesting and planning the present investigation and for their valuable guidance, encouragement and suggestions.

The author is so grateful to Dr.C.K.N.Nair,M.Sc., Ph.D.(Cornell), D.R.I.P. (Oak Ridge), Principal & Additional Director of Agriculture (Research) for his encouragement and also for providing all facilities for the successful conduct of this investigation.

He expresses his sincere thanks to Shri V.Gopinathan Nair, M.Sc.(Ag.), Lecturer in Agricult Botany for his constant and generous help.

He is thankful to Shri E.J. Thomas, M.Sc., (Iowa), Junior Professor in Statistics for his v advices and suggestions.

The author is grateful to all his colle friends for the valuable help rendered by them in t ful conduct of the present investigation.

K. K

C O N T E N T S

INTRODUCTION

INTRODUCTION.

It remained for Muller (1927) and Stadler (1928) to contribute one of the epoch making discoveries to the science of genetics when they propounded independently that X-rays could be used to induce mutations in living organisms. This discovery gave an impetus to increased researches in fundamental genetics. Extensive researches carried out in the past thirty years proved beyond doubt that induced mutations provide an efficient and important tool in Plant breeding. Barring the few limitations and defects inherent to this method, mutation breeding through ionizing radiations envisages an important crop improvement method and play a major role in the present day Plant breeding.

Cereal plants have figured importantly in the development of mutation work since its inception, for it was in barley (*Hordeum vulgare* L.)that Stadler in England and Nilsson-Ehle and Gustafsson in Sweden first induced mutations by means of ionizing radiations. Promising results have been obtained by radiation research in many economic crop plants by various workers.

Gregory (1956) by his comprehensive experiment on ground nut (*Arachis hypogaea*)demonstrated the validity of induced mutations in crop improvement through ionized radiations.

Other examples of mutation breeding by ionizing radiations include the transfer of genes for leaf rust resistance from Aegilops umbellulata to cultivated wheat (Triticum vulgare) by Sears (1956) in U.S.A.; induction of awning in the New Pusa strains of wheat at the Indian Agricultural Research Institute, New Delhi; production of the speltoid barley by Gustafsson (1947) in Sweden, and the like.

Cow pea (Vigna sinensis L. SAVI) constitutes one of the prominent members of the pulse family in India. It is a naturally self-pollinated leguminous plant and therefore, affords very little genetic variability under natural conditions. If, by artificial means genetic variability is induced like that produced by ionizing radiations, selection of better yielding types becomes easy and economic. With this in view, Nair (1964) irradiated the seeds of the 'African' variety of cow pea with X-rays. The X-irradiated seeds were raised in X_1 generation from which five plants which showed seed colour changes were selected. The seeds from these five plants and a sample consisting of 125 seeds from each treatment, were carried forward to the X_2 generation. Various morphological as well as physiological mutants were obtained. It was observed that among the various characteristics studied, seed colour was the most easily affected one. The author selected from this X_2 generation twenty one distinct mutant seed types based on their seed coat colours.

It is the intension of the present investigation to probe in to the details of the morphological and the cytological behaviour of these twenty one mutant seed types during their X_3 and X_4 generations. This also aims at the study of the breeding behaviour of these mutants particularly with respect to their pod and seed colours. It is also, however, desired to isolate distinct pure breeding geno-types from the X_3 and X_4 variable populations.

---:oOo:---

REVIEW OF LITERATURE

REVIEW OF LITERATURE.

1. Germination.

Maldiney and Thouvenin (1898), just after three years of the discovery of X-rays by Roentgen, found that in *Convolvulus* and *Lepidium* germination was hastened by irradiation. A similar result was obtained by Pfiffer and Simmermacher (1915) on *Vicia faba* but Ancel (1924) obtained contradictory results that she could not find any hastening of germination by irradiation. Kumar and Joshi (1939) reported that X-irradiation was found to be deleterious to germination in *Brassica juncea*, *Nicotiana tabacum* and *Pennisetum typhoides*.

Jacob (1949) reported a higher germination percentage in irradiated seeds of Jute (*Corchorus sp.*) He also stated high survivability of plants in some varieties which were irradiated. Spencer and Cabanillas (1956) working with *Indigofera endecaphylla*, reported that X-ray irradiation appeared to promote earlier germination and that seedlings displayed no definite lethal effects, but Lesley and Lesley (1957) reported considerably reduced germination in irradiated tomato seeds. Similar results were obtained by *Matsura et al* (1957) in wheat seeds.

Gottschalk and Scheibe (1960) reported that the germinability of seeds of plants belongining to Leguminosae was independent of X-ray dose which was supported by the findings of Kundu *et al* (1961) in Corchrus *sp*. Similar reports came from several workers as Ahasthry and Ramiah (1961) in *Oryza*, Jain *et al* (1961) in Chrysanthemum, - Sjodin (1962) in *Vicia faba*, and Katayama (1963) in *Oryza-sativa*. Nair (1964) reported that germination was unaffected in dry X-irradiated seeds of Cow Pea (*Vignasinensis*) but irradiation was found to affect germination in soaked seeds. In the X_2 generation of certain mutants, reduction in germination was reported by him.

2. *Growth habit*.

Various workers reported changes in the pattern of growth in different crops in the segregating generations after X-ray irradiation. Gelin (1954) reported multi-branched robust mutants of irradiated *Vicia faba* during the X_2 generation. Bifurcate, concave asymmetric or funnel shaped leaves and bifurcate shoots were observed by Zwintzscher (1955) in the induction of mutant in fruit breeding. Down and Anderson (1956) obtained a bushy type of mutant induced by X-ray irradiation of the Navy bean which had a spreading habit. Wohrmann (1956) induced trailing habit in non-trailing types of *Alopecurus pratensis* by X-ray irradiation.

Vettel (1959) reported X_2 and X_3 mutants of wheat x rye (Triticale) hybrids with abnormal growth habit.

Jain *et al* (1962) obtained twelve X_2 progenies from X-ray irradiated tomato which showed variable growth habit. X_2 mutants of *Cicer*, characterised by small internodes and closely packed leaves and leaf lets, were observed by Athwal (1963).

Narahari and Bora (1963) observed in X_2 generation rice mutant forms with short culms. Nair (1964) grouped the various growth patterns of X_2 mutant cow pea under various heads. Unlike the erect growing 'African' variety, the mutants showed various changes in growth habit and were grouped as twining types, profusely branching and spreading types, dwarf mutants and straight stemmed mutants.

3. Flower Characteristics.

Interesting flower colour changes were observed by various workers in the segregating X-ray irradiated materials. Early in 1930 Goodspeed observed flower colour changes in the progenies of irradiated tobacco. Bruns (1954) reported flower colour changes in *Trifolium* and similar results were obtained by Hoffmann and Zoschke (1955) in Linnum, and by Mehlquist (1957) in Carnation.

Rai and Jacob (1956) isolated a white flower coloured mutant in the X_3 generation of *Sesamum*. They also obtained white flowered mutants in Mustard in the X_2 generation.

Nair (1964) reported flower colour changes from light purple of the 'African' variety (control) to pure white and to yellow in the X-irradiated Cow Pea. Among these three groups, various intermediate forms and colour combinations were noted.

Krishnaswamy (1945) found floral abnormalities including the occurrence of double standards, wing petal-like growth from the androecium, dedoublement of the single separate stamen and increased or decreased number of floral parts in one variety of Cow Pea (Vigna ugniculata L. Walp.)

Later in 1961 Sjodin observed mutants characterised by short corolla tubes, abnormal petals and deformed stigmas in the X_2 generation of Vicia faba. Double flowered mutants were reported by Jain et al (1961) in the X_2 generation of Chrysanthemum. Jain et al (1962) working on tomato obtained plants in X_2 generation with changes in floral structure consisting of an increase in the number of sepals, petals and stamens and thickening and flattening of the style. Bhatia and Swaminathan (1963) reported a multiple carpel flower mutant in the segregating generations of irradiated bread wheat.

Jagathesan and Shasthry (1963) obtained twisted and divided style in Gossypium hirsutum in the X_2 generation after X-irradiation.

Tedin and Hagberg (1952) reported one mutant in the X_4 generation of X-rayed Lupin with reduced petals and with pale greenish yellow colour and the flowers never opened. Hackbarth (1955) found in the X_2 of X-irradiated Lupinus luteus, a plant with floral abnormalities leading to a high degree of physiologically conditioned sterility.

A spontaneous male sterile mutant in Vigna-sinensis catjang "poona" was reported by Sen and Bhowal (1962). The plant was vigorous but had reduced floral parts. A single gene pair involved homozygous recessivity and heterozygosity causing vigorous vegetative growth.

Working with radiation induced mutants in Chrysanthemum, Rana (1964) observed that breakdown of the tubular effect occurred only in the mixed tubular types and plants with perfect tubular flowers appeared quite stable with respect to their flower phenotype.

4. Chlorophyll Mutants.

Various types of chlorophyll mutations were recorded and identified by Gustafsson (1940) in the segregating populations of X-rayed barley. They included types such as 'Albino', 'Xantha''Virescens'and'Chlorina.' Such mutations were also found in barley by Freislben and Lein (1943) Froier (1946).

Gustafsson (1947) grouped the various types of mutants observed in the segregating generations of barley as:-

1). Chlorophyll mutants,

2). Sterility and lethality mutants of different types and

3). Vital mutants - to include both morphological and physiological mutants.

Tedin and Hagberg (1952) observed that the most common type of mutant in the X_3 generation of Lupinus-luteus was chlorophyll defectives. They found two 'chlorinas' in the X_4 generation which were normal green in the rosette leaves, but the apical leaves of the stem were slightly chlorotic. Matsumura and Fujii (1955) reported chlorophyll deficiencies in the induced mutants of N. tabacum and N. sylvestris. Zwintzscher (1955) in his work on the induction of mutants as a method of fruit breeding, obtained forms with chlorophyll deficient in X-ray irradiated populations. Chlorophyll deficient mutants were reported in the X_3 generation of X-ray and thermal neutrons irradiated rice by Beachell (1957).

Carpenter (1958) obtained 42% chlorophyll mutants in the Subterranion Clover. Korah (1959) grouped the various chlorophyll deficiencies observed in the X_1 and X_2 generations of X-irradiated Oryza as - Xantha or Lethal yellow mutants, and Lutescents. These were characterised by the absence of chlorophyll and subsequent death of the mutants.

Vettel (1959) could isolate chlorophyll defect forms from the X_2 and X_3 generation of Triticale (wheat x rye) hybrids. Blixt (1961) identified a type of chlorophyll variegation in X_2 and X_3 of Peas. which has been named as 'chlorotica vario maculata' characterised by patches of green and yellow spots. It was Sjodin (1961) who reported that chlorophyll mutations were comparatively rare in the segregating generations of leguminous plants. He observed, the 'viridis' characterised by different shades of green to be the most common type.

Marki <u>et al</u> (1962) reported that 19% of the X_3 plants of X-irradiated soyabean were albina mutants. Patil and Bora (1963) observed that X-ray induced mutants included an X_3 Xantha mutant and some X_4 virescent mutants in groundnut. A total of nine albino and seven Xantha seedlings were noted by Nair (1964) in the X_2 generation of Cow Pea. He stated that a number of seedlings with chlorophyll spotting on the first leaves were observed. The spottings varied in intensity and extent.

5). <u>Morphological Mutants.</u>

a. <u>Leaf types.</u>

Crinkled and puckered leaves were reported by Goodspeed (1928) in the segregating of irradiated Tobacco. Horlachar and Killough (1931, 1932,) in cotton obtained mutants with forked leaves which were inherited as a simple recessive to the normal. Gustafsson (1947) working with barley observed broader and narrower leaved mutants in the X_2 and X_3 generations. Krishnaswamy (1945) reported tetra-foliate and rarely penta-foliate leaves in a few varieties of Cow Pea. Jacob (1949) found gigantic plants in X-rayed Corchorus plants. Tedin and Hagberg (1952) observed a mutant in Lupinus luteum with deformation of leaf-lets.

An interesting unifoliate mutant in the X_2 of Vicia faba was obtained by Sjodin(1962) with simple leaves instead of the normal trifoliate leaves. Such unifoliate mutants have also been reported by Scheibe and Gottschalk (1956) and Gottschalk (1958). Sjodin (1962) observed one plant with abnormal leaves in which the leaf-lets and stipules were narrow and pointed instead of the normal oval shape, in X_3 irradiated Vicia faba. Athwal (1963) reported narrow and small leaved mutants in Cicer. Many crinkled and distorted seedlings were noted by Nair (1964) in the X_2 generation of X-irradiated Cow Pea. He recorded one'large'leaved mutant in X_2.

Shasthry and Nadhachary (1965) reported rolled-leaved mutants in the X_2 - X_4 generations of X-irradiated rice (NP.130). These plants exhibited an array of phenotypic abnormalities in various plant parts.

b. Early Mutants.

Chaudhuri (1953-1955) observed five early mutants in irradiated Linum, but no plants in the X_2 and X_3 generations were as early as these mutants. Onnfrijchuck (1953) reported that a genetically stable change affecting maturity had occurred in one speltoid mutant since X_2 and X_3 plants ripened several days earlier than the controls.

Matsumura and Fujii (1955) reported a mutant form flowering two weeks earlier than the control among the X-ray induced mutants of Nicotina tabacum and N. sylvestris. Earliness in flowering by seventeen days in mutant forms in the X_3 generation of Sesamum was observed by Rai and Jacob (1956). Similar results were obtained by Gladstones (1958) in Lupinus digitalis. Ehrenberg (1961) obtained forty three early mutants in the X_3 generation of barley subjected to ionizing radiations and chemical mutagens.

But Vettle (1959) observed among the Triticale (wheat x rye) hybrids, fewer early and late forms during the X_2 and X_3 generations. Similar results have been obtained by Abrams and Vélez - Fortuno (1962) who reported early, intermediate and late flowering lines in the X_3 and X_4 generations.

c. Dwarf Mutants.

In the X_3 generation in rice Parthasarathy (1938-1939) observed dwarf plants which produced only semisterile plants while the normal fertile plants bred true. He stated that 'stumpy' and 'beaked sterile' did not breed true and were found to be (2n + 1) types. Tedin and Hagberg (1952) reported dwarf mutants in *Lupinus luteum* in X_3, usually accompanied by other characterestics indicative of a general disturbance of balance. These dwarfs appeared irregularly and in no cases studied there has been even a semblance of normal Mendelian segregation.

Hackbarth (1955) observed in X_2 irradiated *Lupinusalbus*, a number of dwarf plants with short stems. A dwarf mutant was also obtained from *L. angustifolius*. He also reported that changes in growth period were observed in all, especially in the X_3 of *Lupinus luteus*. Such similar dwarf mutants were reported by Matsumura and Fujii (1955) among induced mutants of *N. tabacum* and *N. sylvestris*. Beachell (1957) observed that most of the mutants in the X_3 generation of X-ray and thermal neutron irradiated rice were smaller than normal.

Carpenter (1958) reported that, of the X_2 mutants in subterranean Clover, 28% were dwarf mutants. Quang and Chang (1959) showed that many dwarf mutants of the X-irradiated rice, in back crosses, were shown to be due to a single recessive gene. Korah (1959) observed dwarf mutants in the X_1 and X_2 generations of X-irradiated *Oryza*. She found that these dwarf mutants bred true in X_2 and were also characterised by a high degree of 'Spike-let sterility'.

Nair (1964) founddwarf mutants in X_2 of X-irradiated Cow Pea. Shasthry and Nadhachary (1965) who worked with X_4 generation of irradiated NP. 130 rice suggested that dwarfs are the most frequent of all viable mutations.

6. Fruit Characteristics.

Gustafsson (1947) reported erectoid mutants in the segregating generations of barley which were characterised by very compact ears with projecting kernals. Rai and Jacob (1956) obtained smaller seeded *Sesamum* mutants in the X_4 generation. Rai (1959) isolated mutants with narrow slender and erect pods in the X_2 segregating generation of *Brassica*. The same author in 1959 described a mutant with thickened pods in X-ray irradiated *Brassica-nigra*.

Changes in the position of fruits in X-ray irradiated *Vicia faba* were reported by Lechner (1959). Sjodin (1961) pointed out the occurrence of plants in the X_2 of *Vicia faba* with glabrous pods unlike the normal plants with hairy pods. He also reported long and narrow pods in the advanced generations. Jain *et al* (1962) found uniform ripening fruits of *Lycopersecum esculentum* in four of the X_2 populations. The plants showed reduced fruit set and changes in fruit shape.

Nair (1964) found many variations in the X_2 generation of Cow Pea, mainly those affecting the size and colour of pods. Colour variations included blackish, brownish, reddish white, reddish white with deep purple strêks and light green colours, the control being straw coloured. Size of pods varied from large to small.

7. Seed Colour changes.

A large number of seed colour mutants were observed by Stadler (1931) in Zea mays and these were found to be simple recessives. (1) Zachow (1958) obtained mutant plants in the X_2 and X_3 generations of irradiated Lupinus luteus having smaller seeds. (2) Papa and Williams (1959) reported small and large sized seed mutants in the X_3 irradiated population of soyabean.

Smaller, smoother, and light coloured seeds obtained from the X_3 population of X-ray irradiated Sesamum were reported by Nair (1961). The mutant characters were found to breed true in the X_4 generation. Almost all the induced mutants in Vicia faba that Sjodin (1961) found were concerned with seed coat colour. He isolated fifteen mutants affecting the seed coat colour from the X_2 population. It was observed that some of these colour changes were associated with other characters like earliness, growth habit, etc.,

Seed colour mutations in rice were reported by Nishimura and Kurakami (1952) and Campos and Espiritu (1960) Nair (1964) obtained five plants from the X_1 germination of irradiated Cow Pea with colour variations. These plants, together with other selected mutants segregated for twenty one seed coat colours during the X_2 generations.

8. <u>Induced variability</u>.

Marked variation in the X_2 and X_3 progenies of X-ray irradiated progenies of Peanuts was observed by Gregory (1955). Bora and Rao (1960) obtained a wide range of variation of characters such as seed sterility, albino seedlings, dwarfing, time of flowering etc., in the X_3 generation of rice subjected to ionising radiations. Kato <u>et al</u> (1960) stated that reduction in variability in the X_3 population of irradiated rice may have been largely due to the result of natural elimination of unadapted mutations in the X_1 and X_2.

Jain <u>et al</u> found in the X_2 population of Tomato, induced variations including a macromutation affecting leaf, growth habit and flower form. They stated that the induced variation in respect of ripening of fruits and other characters like locule number was found to breed true in the X_3 generation. In the X_3 generation of X-ray irradiated Soyabean, Marki <u>et al</u> (1962) observed variability in morphology and yield characters. Palenzona (1962) pointed out a significant increase in variability in the X_3-irradiated <u>Triticum aestivum</u>. He concluded that the increase in variability is due to mutations in a multifactorial system rather than in the major genes. Rana (1964) working with radiation induced mutants in <u>Chrysanthemum</u> suggested that the variable expressivity is primarily a function of the variable genotypic background.

9. Sterility Mutations.

Sterility mutants are offrequent occurrence in segregating populations after X-ray irradiation. Some such plants which failed to flower were reported by Gustafsson (1947) in barley and Sjodin (1961) in *Vicia faba*.

Tedin and Hagberg (1952) observed a fairly high frequency of partially sterile plants in the X_2 and X_3 generations of X-ray irradiated Lupin. They reported that one of the sterile types in X_2 had reduced petals but with normal pollen. These workers obtained four distinct mutations with complete functional sterility in the homozygote combined with vegetative vigour. In the X_2 generation of X-ray irradiated *Hordeum vulgare*, Das (1955) observed a number of semisterile plants characterised by high pollen and ovule abortion. Beachell (1957) stated that mutants obtained from X-ray and Thermal neutrons - irradiated rice showed considerable sterility during R_1 - R_3 generations. Results of a similar nature have been obtained by Ouang and Chang (1960) who noted varying degrees of sterility in the progenies of irradiated rice in X_2, X_3 and X_4 generations. In the X_4 generation of wheat Bozzini (1961) reported that the degree of sterility varied independently of the degree of meiotic abnormality.

Carpenter (1958) observed in X_2 progenies of subterranian Clover that, complete fertility was shown by 31%

while 41% were classed as semi-sterile and 28% as fully sterile. ⑥Contradictory to the above findings, Vettel (1959) reported that mutants in the X_2 and X_3 generations of Triticale hybrids were superior to the original form in fertility. Jain et al (1961) found that pollen and seed fertility in the X_3 generation of Chrysanthemum was not found to be very much affected. So also in X_4 most of the plants showing altered flower forms had over 80% pollen fertility compared to 95 - 100% in a corresponding group of controls.

⑦ X_3 progenies of tomato within a culture showed variation in the fertility of their seeds. This was reported by Jain et al (1962). Athwal (1963) stated that the sterile mutants he obtained in Cicer had luxuriant vegetative growth. Two types of steriles were reported by him, in one the flowering was normal but the anthers failed to dehisce and the pistil deformed where as the other was with highly abnormal flowers. Nair (1964) reported two sterile mutants in the X_1 of X-ray irradiated Cow Pea, one plant failed to flower, and the other flowered but failed to set fruits.

10. Cytological abnormalities.

Parthasarathy (1938-1939) described the genetical and cytological behaviour of 3 mutants from X_3 generations of X-ray irradiated rice. Cytological examination of these semisterile plants in X_3 showed that they contained the normal complement of chromosomes and the meiosis was regular although they did not set seeds. The origin of this mutants may probably be due to a small deficiency or a gene mutation.

Cytological studies of a number of semisterile plants in the X_2 generation of Hordeumvulgare by Das (1965) revealed structural changes characterised by translocations and paracentric inversions in the chromosomes and frequent chromatin bridges and acentric fragments at meiosis in the pollen mother cells. Matsumura and Fujii (1955) reported chromosomal aberrations including translocations, univalents, fragment formation and asynaptic configurations and mutations resulting from X-ray irradiated Nicotiana sylvestris and Nicotiana tabacum.

Onnfrijchuk's (1953) cytological analysis of one speltoid mutant in barley in X_3 revealed that this mutant had 21 pairs of chromosomes +1 iso-chromosome or 20 pairs + 3 isochromosomes. It is assumed that in the production of this X_3 mutant, at least two reciprocal translocations had occurred. Ouang and Chang (1959) observed in pollen mother cells of semisterile progenies obtained from X-ray irradiated rice, both quadrivalents and univalents at diakinesis and metaphase I. But Vettel (1959) could not find any relationship between fertility and the percentage of normal configurations in meiosis in X_2 and X_3 of Triticale hybrids subject to repeated irradiation in successive generations.

A study by Bozzini (1961) of the R_3 generation of wheat previously irradiated showed the presence of reciprocal translocations in three plants. In the R_4, a line with $2n = 26 + 1$ was isolated and associated anomalies were also noted. He suggested that the degree of sterility raised independently of the degree of meiotic abnormality.

11. <u>Segregation of characters.</u>

Levan (1944) obtained three families in X_2 of flax which segregated for chlorophyll deficiency. Of the 95 X_3 families raised, no progenies of the mutant homozygote were present, thirty one progenies were constant and normal while sixty four segregated into normal and mutants in the expected 2:1 ratio. Further, it was observed that the distribution of mutants over X_3 families strongly suggests at least two classes, one with one mutant and the other with between four and twenty-one mutants.

Fujii (1955) reported chlorina mutants in <u>Triticum - monococcum</u> obtained by X-ray irradiation was found to be a simply inherited recessive character. Similar results have been obtained by Matsumura and Fujii (1955) in irradiated <u>T. monococcum</u>, who stated that the chlorophyll and other types of mutans obtained from the X_2 were found to be recessive and monogenic in inheritance.

In X-ray irradiated Lupinus leteum, Tedin and Hagberg (1952) observed that one of the chlorina mutant gave approximately 3:1 segregation. Hackbarth (1955) reported that in the case of the three X-ray irradiated Lupinum sp, changes in leaf colour appeared to depend upon a single recessive gene.

Bora and Rao (1960) working with X_3 rice suggested that heterozygous deficiency for a segment carrying an inhibitor for round grain is held to be responsible for X_3 segregation for long grained: round grained plants in a 3:1 ratio. X_3 segregations of 1:63 and 1:3 for albina : normal plants were also interpreted. X-ray induced chlorophyll mutants obtained by Patil and Bora (1963) in ground nut segregated in X_4 and X_5 for normal and virescent types in ratios ranging for 1:1 to 15:1 indicating that the development of chlorophyll is possibly controlled by more than one locus.

Some selection of irradiated Corchrus olitorus and C. capsularis segregated into normal and morphologically aberrant types in the X_3 and X_4 generations. (Anon. 1955, 1956) Ouang and Chang (1959) observed that many dwarf mutants of X-ray irradiated rice were shown in back crosses to be due to a single recessive.

Data collected by Murray and Craig (1962) from X_2, X_3 and X_4 segregating lines in Medicago sativa demonstrate that two closely associated characters viz.

cauliflower head and single leaf are determined by a single recessive gene inherited tetrasomically.

-: oo 0 oo :-

MATERIALS AND METHODS

MATERIALS AND METHODS.

Experiment.

The present investigation aims at the "Studies on the morphological and cytological behaviour of X_3 and X_4 generations in cow pea (*Vigna sinensis* L.SAVI)", variety 'African'. The various morphological characteristics of the mutants mainly of a qualitative nature were studied combined with cytological studies.

Experimental site and Layout.

The work was carried out in the Agricultural Botany Division, Agricultural College and Research Institute, Vellayani, Kerala; during the academic year 1964-65.

Simple field trials were followed in both generations of X_3 and X_4. The entire area was divided into three blocks with twenty four beds (15' x 3') each, making altogether seventy two beds. X_3 generation was raised in these beds. During the X_4 generation twenty five beds (25' x 3') were taken and seeds sown in these beds. Cultural and manurial applications were uniform in all beds during the course of the experiment.

Seed Material.

The material for the study consisted of twenty one different mutant seed types obtained from the X_2generation

TABLE I.

X_3 Seed characters.

Seed type numbers.	Number of seeds.	Seed types.
i.	133	Control--African variety of Cow Pea.
ii.	180	White seed type.
iii.	67	White with brown patch around the eye.
iv.	135	White with bluish mottling around the eye.
v.	66	White with Red patches around the eye.
vi.	24	White with black patches around the eye.
vii.	575	Reddish with black mottlings.
viii.	360	Deep brown mottled.
ix.	180	Light brown mottled.
x.	180	Reddish white with brown mottlings.
xi.	90	Yellowish white with "light brown mottling.
xii.	90	Grey--ash mottled.
xiii.	40	Large brown.
xiv.	370	Small brown.
xv.	90	Brown medium seeds.
xvi.	38	Reddish white.

(continued)

of irradiated cow pea studied by Nair (1964). The material was first irradiated with different doses of X-rays from 1,000 r to 15,000 r using a Philips X-ray unit at the Agricultural College and Research Institute, Coimbatore. Each seed lot was divided into two groups viz. dry and soaked and 8 doses with 16 treatments were given. The present work therefore aims at studying the X_3 and X_4 generations with the twenty one selected mutant seed types.

a) Seed Material for X_3

The twenty one mutant seed types isolated in X_2 were carried forward in the X_3 generation. The seed characters of these X_2 types are presented in Table.I. These twenty one seed types consisted of 2,964 seeds all of which were sown, different types being sown in different beds.

During the harvest of the X_3 generation, individual plants were harvested separately. The plants raised from each type were classified into different groups based on their pod and seed colours. Types which show segregation and true breeding, are grouped separately and tabulated.

b) Seed Material for X_4 generation.

The seed material for the X_4 generation included segregants obtained from three X_3 seed types which showed discernible segregation into different seed and pod characteristics.

TABLE I. (contd).

Seed type numbers.	Number of seeds.	Seed types.
xvii.	24	Half-red half-white type.
xviii.	12	Deep violet mottled.
xix.	80	Deep ash mottled.
xx.	45	Greyish mottled large seeds.
xxi.	113	Brown mottled.
xxii.	72	Light yellowish small seeds.

I) Seed type ii in X_3 - White seeds.

The following seed types were selected from the segregants:-

1. Straw White (Parental type)
2. Straw Brown
3. Purple White
4. Purple Brown, and
5. Pink White.

2) Seed type iv in X_3- White with bluish mottlings around the eye.

Following seed types were selected from the segregants:-

1. Straw White (Parental type)
2. Straw Brown mottled
3. Straw Ash mottled
4. Straw Brown, and
5. Pink White.

3) Seed type x in X_3 - Brown mottled.

Following seed types were selected from the segregants:-

1. Straw Brown mottled (Parental type)
2. Straw Rose white
3. Straw Brown, and
4. Straw White.

Accordingly there were fourteen seed types which were obtained from three X_3 types and were carried forward to the X_4 generation. Depending on the total number of plants in each group one, two or three plants in the same group were selected for the X_4 generation. A total of 25 plants were selected to represent the various fourteen X_3 segregant lines from the three seed types. While sowing, seeds from each pod of each plant were sown separately without mixing them. 48 seeds taken either from one, two or three plants in equal numbers, were sown.

Apart from the characters viz., pod colour and seed colour, some other mutant characters were noticed in the X_3 and selections of these mutant types and their carrying forward to the X_4 generation were also made. They included,

1) <u>Abnormal flower type.</u>

One plant obtained from the type ii in X_3 was with variation in the number of floral parts as well as variations in their disposition. All the pods of this plant were studied in pot culture in the X_4 generation.

2) <u>Large Leaved Mutant type.</u>

Out of thirteen such large leaved mutant plants, one plant was selected in X_3 and carried forward. This type was isolated from the seed types xiv and xv of X_3 and 48 seeds were sown in X_4.

3) Crinkled plant type.

One pod obtained from a plant in the seed type (Small brown) in X_3 and seeds were sown in pots.

OBSERVATIONS TAKEN.

During the course of the investigation of X_3 and X_4 individual plant characteristics were studied. The following were the observations taken.

1) Germination percentage.

The germinated seedlings in the field were enumerated from the 3rd day onwards of sowing and continued until the 7th day in both the trials and data were used for analysis.

2) Percentage of survival.

Counts of the survived plants were made at the time of harvest and the data tabulated.

3) Chlorophyll variations.

Fewer cases of chlorophyll variations found in the X_3 population such as lethal, xantha mutants etc. were observed and some carried forward to X_4 generation. The data are tabulated.

4.) Leaf characteristics.

Second leaf characteristics such as bifoliate, tetra or penta foliate in lieu of the normal trifoliate leaves were observed and recorded.

5) Growth habit.

The natural growth tendency of each mutant type, either bushy or trailing, was noted in both generations. Flowering and flower types were also studied.

6) Pollen sterility.

Sterility studies in X_3 were done in a random sample of 5 plants from each bed. The pollen grains were stained with glycerine--acetocarmine and sterility counts were made from 50 microscopic fields in each type and the data were used for calculating the percentage of sterility in each type.

In the X_4 generation, sterility studies were made in individual plants in each type, as in the previous case. In each type, 10 plants were studied within a progeny line and the data recorded. Separate counting was done among plants of a line as well as within one plant for different flowers, in the case of the "Floral-abnormality" type. Studies between individual plants were also made in two other types viz., the "Large leaved" mutant type and the "Crinkled mutant" type and data recorded.

7) Morphological abnormalities.

a) Floral-abnormality type.

The progenies of one plant isolated in X_3 which showed variations in the number of floral parts as well as in their arrangements were carried forward to X_4 and subjected to various studies.

b) <u>Large leaved Mutant type.</u>

One of the plants, observed to be contrasting in many of its morphological characteristics, was carried to the X_4 generation and various studies were conducted to determine whether it is a polyploid.

8) <u>Colour of pods.</u>

The colour of the unripe pods was studied both in X_3 and X_4 generations. The pod colour was either green, pink or shaded.

9) <u>Colour of seeds.</u>

Importance was given in the study of the behaviour of seed colour in the two generations. Classification of seed types in combination with pod colour was made in X_3 and segregation or pure breeding nature of these types studied. Among the various types of X_3, three groups were carried forward to X_4 for further studies.

10) <u>Association of characters.</u>

a) Association of characters between colours shades on the surface and tip of the pod were observed during X_3 and X_4 generations.

b) Between flower colour and seed colour while flower and seed colours in some types were studied in X_3 and X_4 generations.

11) <u>Weight of 100 seeds.</u>

The seed weight of X_2 seed material was taken initially. Later seed weight for the different parental and segregating classes of X_3 was taken. In X_4 the seed weight of the progeny materials was also taken, data recorded and analysed.

12) <u>Cytological studies.</u> Cow pea 2n = 22.

Cytological works consisted of studies of the stages of mitosis and meiosis. Cow pea is characterised by its very small chromosomes which rendered difficulty in handling the material in this investigation. Moreover, the cytoplasm in the pollen mother cells imbibed much stain which also was a handicap in meiotic studies to get good preparations.

For mitotic studies, five seeds each of the different seed types were germinated in Petri dishes on moist filter paper. When the roots were about two cm. in length, root-tips were fixed in 3:1:1 (alcohol, acetic acid and chloroform) between 12 noon and 2 pm. Squashes were prepared in Haematoxylin following Heidenhains method. The procedure adopted was,hydrolysing the root tips at 60°C in NHcl for 15 minutes, mordanting in 4% Ferric ammonium sulphate and staining in 0.5% haematoxylin solution followed by squashing in 45% accetic acid.

Various mitotic stages obtained were studied. Meiotic studies were also conducted for all seed types especially for the large leaved mutant type. The method followed was-fixing young flower buds in acetic alcohol (1:3) between 11 am. and 1 pm. Smears were made from these buds using 1% Propionocarmine and various stages studied. Camera-lucida drawings were also made for some of the stages.

......

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS.

Results of the present investigations on the morphological and the cytological behaviour of the X_3 and X_4 generations in Cow Pea are presented below:-

I. Observations on the X_3 generation.

1. Germination.

Observations were taken from the fourth day onwards after sowing. Good germination was found in types ii, vii, x, xi, xvi, and xviii on the fourth day and by the seventh day it was complete in all types. The percentages of germination for different seed types are given in Table.II.

Type No. vi was completely inviable and produced no plants. Delayed germination was observed in some seed types particularly in iii, xvii, and xxi.

2. Seedling characteristics.

i) First pair leaves.

Table III illustrates the first leaf characteristics. The first visible observation recorded was that of the appearance of four yellow seedlings. Abnormal seedlings were found in types viii, x, and xiii, having one seedling each in these types.and the seedlings later produced normal leaves. Observations were made for those seedlings that were not well developed compared to the

TABLE II

Table showing 100 seed weight, germination percentage and percentage sterility of the different seed types in X_3

Type No.	Percentage generation	Percentage sterility	Seed weight (gm.) (X_3) seed material (100 seeds)
i	80.05	22.45	6.75
ii	84.44	39.39	9.13
iii	74.53	31.48	8.44
iv	97.04	34.31	8.57
v	57.57	31.03	6.82
vi	-	-	12.92
vii	86.61	20.51	8.40
viii	68.61	32.56	8.61
ix	77.77	28.42	8.72
x	85.55	16.71	7.70
xi	77.77	18.30	7.82
xii	90.00	30.54	7.56
xiii	57.50	29.77	13.32
xiv	84.32	26.42	9.45
xv	60.00	24.06	8.58
xvi	94.74	23.84	11.44
xvii	20.83	31.91	9.52
xviii	100.00	20.33	13.30
xix	82.50	17.24	9.92
xx	68.88	23.27	15.76
xxi	56.64	23.82	7.90
xxii	90.02	19.68	6.28

control types. Seedlings with crinkled first pair of leaves were noticed in almost all seed types but more in types ii, iv, viii, xiv and xix. The time of emergence of the first pair of leaves varied in different types as well as in different seedlings in a type. Three seedlings in seed type ii, two seedlings in iii, five seedlings in iv, four seedlings in v, fifteen seedlings in type vii, three seedlings in x, one seedling in seed type xii, one seedling each in seed types xix and xx and two seedlings in seed type xxii - produced the first pair of leaves within 7-10 days after sowing unlike the control which took only 5-7 days for the same.

ii) Chlorophyll variations.

The chlorophyll variations in the first pair of leaves as well as the succeeding leaves consisted of partial absence of chlorophyll, chlorophyll deficient spots, or unevenly distributed yellow and green patches. Completely yellow seedlings were also noticed. Generally chlorophyll variations were observed in the first pair of leaves and rarely in the second pair of leaves also. The frequency of different types of chlorophyll variations in the first pair of leaves is given in Table III along with other seedling abnormalities.

Seedlings with the first pair of leaves partly yellow were noticed in seed types ii, iii, iv, vii, viii, ix, x, xii, xiii, xiv, and xxii.

TABLE III

Characteristics of the first pair of leaves in X_3

Type No.	No. of abnormal plants	No. of plants not well developed	No. of plants with crinkled leaves	No. of plants showing Chlorophyll variations		
				Leaves yellow	Partly yellow	Yellow or white spots
i	-					
ii	-	3	24	-	2	-
iii	-	2	2	-	2	-
iv	-	1	12	-	-	2
v	-	3	2	-	-	-
vi						
vii	-	15	8	-	3	2
viii	1	11	12	-	2	1
ix	-	5	7	-	2	1
x	1	3	5	-	3	1
xi	-	1	1	-	-	-
xii	-	1	8	2	2	-
xiii	1	-	1	-	3	1
xiv	-	7	18	-	2	1
xv	-	4	2	-	-	-
xvi	-	-	1	-	-	-
xvii	-	-	3	-	-	-
xviii	-	-	1	-	-	-
xix	-	2	11	2	-	-
xx	-	1	-	-	-	3
xxi	-	5	8	-	-	-
xxii	-	2	7	-	1	-

Yellow or pale white spottings or markings found in some seedlings under seed types iv, vii, viii, ix, x, xiii, xiv, and xx.

Chlorophyll variations were observed later in the growth period also. One such case was found in seed type ii in which one plant showed three leaves with distinct yellow and white patches. This plant produced normal leaves in the earlier stage but showed chlorophyll variation in the eighth ninenth and tenth leaves. The later formed leaves were normal. Seed type xix had one plant with the second pair of leaves more or less yellowish and the third leaves were partly coloured. The variation in colour gradually decreased in the succeeding leaves.

Chlorophyll spottings were observed in the first and second leaves and rarely in the third and fourth leaves. The frequency thereafter decreased and was completely absent in the later formed leaves. Almost all of these plants survived and produced pods but to a lesser extent.

iii) Lethal 'Xantha' mutants.

In seed types xii and xix, four Xantha mutants two in each type were observed. These seedlings were normal, earlier in germination but were compltely yellow. They remained for about one week and then faded out and none survived.

3. Leaf abnormalities.

This was a common feature of the seedlings during their earlier growth period. Generally the second and other succeeding leaves have three leaflets of equal size and shape. Exceptions to this condition were noticed in many of the seed types. Plate 5 shows such abnormal leaves.

Unifoliate second pair of leaves were observed in one plant under seed type iv.

Seed types ii, iii, iv, v, vii, viii, ix, x, xi, xii, xiv, xv, xix, and xxi produced bifoliate leaves. Four-foliate leaves were produced by some plants in seed types ii, iii, iv, vii, ix, xi and xii.

Four plants under seed types ii one in v and one in ix, produced five foliate leaves.

Many of these cases were modifications or abnormalities of the second leaves of the seedlings. Only very few cases of abnormalities in the third or the succeeding leaves were noted.

4. Growth habit of plants.

In general, all seed types showed a uniform rate of growth like the control type. But there were variations in the final stature of the plants. The plants within a type were found to consist of tall or dwarf, erect or spreading or sometimes trailing. The growth habits of different seed types are summarised as follows. Seed types ii, iii, iv, v, vii, viii, ix, xi, xiv, xv, and xxii were of normal growth as that of the control type I.

TABLE IV

Total Survived plants, Dwarf and Crinkled plants in the different Seed types in X_3

Seed types	Total number of plants in X_3	Dwarf plants	Crinkled plants
i	106	3	-
ii	139	7	2
iii	46	9	3
iv	101	15	3
v	29	5	1
vi	-	-	-
vii	433	82	2
viii	218	70	3
ix	126	24	-
x	140	24	3
xi	60	10	-
xii	76	19	-
xiii	21	6	2
xiv	277	53	4
xv	23	6	-
xvi	27	-	-
xvii	3	2	-
xviii	9	2	1
xix	56	4	1
xx	29	8	2
xxi	41	16	3
xxii	56	8	-

Majority of plants in these types were of medium stature and semi spreading or erect and fewer spreading. Dwarf plants were common in these types, the maximum frequency being in types vii, xiii, ix, x, and xiv which is presented in Table IV.

Medium or tall plants were common in seed types xii, xvi, xvii, xviii xix and xx which were also characterised by a robust or vigorous growth habit. Most of the plants in other types were found to be spreading in nature and dwarf plants were reduced to a minimum in these types.

Seed types xiv and xv were characterised by the presence of plants with large, dark green leaved, later flowering types. These were enumerated to be thirteen (nine from type xiv and four from type xv). In addition to the leaf character which is the most important feature, the robust growth habit combined with shy branching nature and trailing habit were also contrasting. This type was carried forward to the X_4 generation for further studies.

5. Flowering and flower types.

Flowering was first observed in seed types ii, iii and vii on the 34th day after sowing. Flowering started in almost all types within a week and flower production was maximum within a fortnight. Later flowering was observed in types xii, xiii, xvi, xvii, xviii and xix which were about two weeks late compared to the control type. In between these two categories come seed types iv, v, viii, ix, x, xi, xiv, xv, xx, xxi, and xxii.

Control type. Flower colour typical to the 'African' variety characterised by a pink colour, the standard being more intense than the wings. There are two small white spots at the neck of the standard surrounded by a dark hallow from which dark streaks appear to emerge and terminate at the outer perifery. Wings are pinkish with a white patch at the centre.

The flower types produced by the different seed types varied from each other and the different types observed were grouped under nine types as follows:-

i. White flower type.

a) Pure white - This is characterestic of the seed type ii. The flowers were pure white with only a light yellow shade at the neck and were of equal size as the control.

b) White with pink shaded wings - White standard and wings partly shaded. This was produced by seed type iii.

c) White with pink shaded wings and standard - The wings were pink shaded on the upper side close to the standard and this was produced by seed type iv.

ii. Deeply pink shaded types.

The standard was deep pinkish compared to that of the control. Wings were also intensely coloured. Such flowers were noticed in seed types xiii,xviii, xix and xxii.

iii. Pale coloured pink flowers.

The standard was pale coloured with clear hallows at the base. Wings were slightly bluish with a light white patch at the centre. This type was observed in seed types v, vii and viii.

iv. Bluish red.

The standard was slightly bluish red with clear yellow spots at the base while wings were pale coloured. This types was observed in seed types ix,x, xiv and xvii.

v. Small pink flowers.

Both standard and wings were pale pinkish usually with no black hallows around the yellow spots at the neck of the standard and was found in seed types xi, and xv.

vi. Pale coloured with prominent black hallows.

The wings were usually deep pink with a bluish tint at the apex. This was noted in seed types xii and xvi.

vii. Bluish pink coloured

The standard was clearly bluish at the centre and the perifery with no black hallow at the base. Wings were with bluish and pinkish tints at upper and lower areas. This group of lowers was noted in seed type xx and xxi.

Variation of flower colour was observed within many of the seed types. It was not uncommon to find fewer coloured plants in the normally white flower types. So also white flowered plants were observed in many of the coloured types. Seed types ii, iii and iv consisted of some coloured plants while seed types v, vii, viii, ix, x, xi, xiii and xv were found to produce sparse white flowered plants.

6. Pod colour.

The colour of pods produced by the different seed types was studied when the pods were ripe. The different pod colours were recorded and listed out in the Table V along with the seed colours. A close observation of the data discloses that the commonest colour was straw like that of the control type. Variations to this were the purple coloured, pink shaded, and the pink variegated pods. All seeds types produced straw pods. In addition, types ii, viii, ix, x, xi and xiii produced purple pods, ii, iii, iv, v, vii, vii x, xi, xiii and xxii produced pink shaded pods while types xiv and xv had few plants with pink variegated pods. Plates 11 and 12 illustrate the different pod types obtained.

7. Seed colour types.

The seed colours obtained were tabulated and presented in the Table V along with their pod colours.

Plates 1 and 2 represent the parental seed types used as the seed material in X_3. The different seed types obtained from this generation were more or less similar, only three new seed types could be isolated.

It was interesting to note that among the twenty-one

TABLE V.

Pod and seed types obtained from X_3 generation.

I. Control ....'Straw-brown' 36

II. Parental type ....'White seeds'

Segregants

Pod Colour	Seed colour. Parental type	Brown	Total
Straw	65	2	67
Purple.	42	1	43
Pink	20	..	20
Total.	127	3	130

III. Parental type..........'White seeds with brown patch around the eye.'

Segregants.

	Parental	White with out brown patch.	Light brown mottled.	Brown	Total
Straw	20	12	1	1	34
Pink	1	..	..	..	1
Total	21	12	1	1	35

TABLE V. (Contd.)

IV. Parental type ..... ' White seeds with bluish mottlings around the eye'.

Segregants.	Parental type.	Rose white brown mott-led.	Ash mott-led.	Brown.	Total
Straw	61	11	8	3	83
Pink	8	1	..	..	9
Total	69	12	8	3	92

V. Parental type ..... ' White seeds with Red eyes'.

Segregants.	Parental type.	Rose white with-out patch.	Brown.	Total
Straw	20	1	5	26
Pink	1	..	..	1
Total	21	1	5	27

TABLE V. (Contd.)

VII. Parental type ..... ' Red brown with black mottlings'.

Segregants.	(Parental type.)	White with brown patch.	Ash mottled.	Brown.	Total
Straw	61	6	2	5	74
Pink	47	..	..	..	47
Total.	108	6	2	5	121

VIII. Parental type ..... '<u>Red brown mottled</u>'.

Segregants.	(Parental type.)	Rose white.	Ash mottled.	White with brown patch.	Total
Straw	26	7	2	4	39
Pink	3	..	..	..	3
Purple	7	..	..	..	7
Total	36	7	2	4	49

TABLE V (contd.)

IX. Parental type .... Deep Brown mottled.

Segregants.

	(Parental type)	rose white	ash mottled.	White with brown patch.	Total.
Straw	37	3	1	5	46
Purple	1	1	..	4	6
Total	38	4	1	9	52

X. Parental type .... Brown mottled.

Segregants.

	(Parental type)	rose white	Brown	White with Brown patch	Total
Straw	34	7	7	4	52
Pink	1	1	1	..	3
Purple	..	..	1	..	1
Total	35	8	9	4	56

TABLE V (contd.)

XI. Parental type .... Light brown mottled.

Segregants.

	Light brown mottled (Parental)	rose white	Total
Straw	19	..	19
Pink	3	..	3
Purple	25	1	26
Total	47	1	48

XII. Parental type .... Densely mottled dull brown.

Segregants.

	Densely mottled dull brown (Parental)	rose white	dark brown dense mottled	Total
Straw	20	8	7	35
Total	20	8	7	35

TABLE V.(contd.)

XIII. Parental type .... Large light-brown.

Segregants.

	(Parental) large light brown	White	Brown	Total
Straw	10	..	..	10
Pink	3	2	..	5
Purple	5	..	1	6
Total	18	2	1	21

XIV. Parental type .... 'Small brown'.

Segregants.

	Small brown (Parental)	Total
Straw	61	61
Pink(Varigated)	3	3
Total	64	64

TABLE V (contd.)

XV. Parental type - 'Small light brown'.

Segregants.	(Parental) small light brown.	Brown.	Total.
Straw.	32	2	34
Pink (Variegated)	3	..	3
Total.	35	2	37

XVI. Parental type ....'Reddish white'.

Segregants.	(Parental) Reddish white.	Total.
Straw.	25	25
Total.	25	25

XVII. Parental type .... 'Half red- Half white'.

Segregants.	(Parental) Half-red.	Brown.	Total.
Straw.	..	3	3
Total.	..	3	3

TABLE V (contd.)

XVIII. Parental type ....'Ash-violet mottled!

	(Parental) Ash-violet mottled.	Total.
Straw	7	7
Total	7	7

XIX. Parental type ....'Deep ash mottled!

Segregants.

	Deep ash mottled (Parental)	Total
Straw.	51	51
Total.	51	51

XX. Parental type .... Ash-grey mottled.

Segregants.

	(Parental) Ash grey	Rose	Total
Straw	21	4	25
Total	21	4	25

TABLE V. (contd.)

XXI. Parental type .... Brown mottled.

Segregants.

	(Parental) Brown mottled.	Rose white.	Total.
Straw.	31	6	37

XXII. Parental type .... Light yellow brown.

Segregants.

	Light yellow brown (Parental).	Brown.	Total.
Straw.	37	10	47
Pink.	2	..	2
Total.	39	10	49

seed types, only four of them (types xi, xvi, xviii and xix) bred true with respect their pod and seed colours while type xvii produced an entirely different seed types instead of its parental type. All others segregated into different seed colour types. White seed type was the commonest one among the segregating lines.

8. Morphological abnormalities.

a) Floral abnormality.

One plant was isolated from seed type iv which showed abnormalities in the number of floral parts as well as in their order of arrangement. This plant, unlike the control, produced flowers with three standards, two standards or one standard. Other floral parts also showed similar increase or decrease in number or sometimes other structural modifications. In some flowers the staminal column was modified into a triadelphous or tetra adelphous instead of the normal diadelphous condition. Plates 16, 17 and 18 illustrate the various floral abnormalities. In some flowers an increase in the total floral parts was also noticed. This plant was carried forward to the X_4 generation.

b) 'Large - leaved' mutant.

Thirteen plants were selected from seed types xiv and xv and the plate 8 shows one such plant. These plants were characterised by large, dark green broad leaves unlike the control types, which had smaller leaves. These plants had fewer but long trailing branches with a thick and stout main stem. They grew slow and produced flowers by about two weeks later than the control. Flowers were comparatively larger and produced fewer pods perpeduncle. It was interesting to note that some of these plants produced peduncles below the cotyledonary node and above the collar region. Pods were larger sized with irregularly distributed deep pink patches on a straw back ground.

These plants were found to be longer duration than other types. On selfing five fruits were secured which were used as seed material to raise the X_4 generation. Plate 12 (x) shows the pod characteristics of this type.

9. Pollen Sterility.

Pollen sterility was estimated from a random sample of plants, the size of the sample being proportionate to the total number of plants under each type. The data are included in Table II.

The data reveal that there is considerable variability in sterility in the different seed types in comparison to the control. It was highest in the seed type ii and lowest in x.

Pollen sterlity of individual plants was calculated for the'Abnormal floral'type and 'Large leaved' mutant type and data tabulated in Tables IX and X.

II. Observations in the X_4 generation.

1. Germination.

The data representing the germination percentages of the three parental seed types and those of their progeny lines are tabulated and presented in Table VI.

Similar to the parental types, the seeds of the different progeny lines germinated from the third day after sowing and continued for one week. Delayed germination was noticed in lines, 13, 14, 21, 22 and 23.

A comparison of the parental percentage of seed type ii with those of its progeny lines shows that the latter excelled in germinability. Complete germination was observed in some lines and none of these progeny lines gave a lower germination percentage than the parental type.

Seed type iv showed very good germination where as its progenies showed varied percentage. There were lines which had germination percentages better than or equal to or lower than that of the parental type. Type x also simulates type iv in this respect.

2. Flowering and Flower types.

Flowering started by the fifth week after sowing in all lines in the X_3 parental type ii which flowered on the 34^{th} day of sowing. Early flowering was noticed in all lines of the type ii and those in types iv but lines in type x were slightly late.

TABLE VI.

Comparison of the germination percentages and Flower types of the X_3 parental types with those of the X_4 progeny lines.

X_3 Parental types.	X_4 progeny lines	Germination percentage.	Flower colour 'white'	Flower colour 'pink'	100 seed weight (Gms)
	1	2	3	4	5
ii White seed type.	...	84.44	127	3	8.40
	1. Straw white.	100.00	16	..	8.23
	2. ,, ,,	100.00	13	2	..
	3. ,, ,,	100.00	14	..	..
	4. Straw brown.	91.66	6	17	9.62
	5. ,, ,,	100.00	3	17	..
	6. Purple white.	94.66	24	..	7.40
	7. ,, ,,	95.83	22	..	..
	8. Purple Brown.	91.66	7	34	6.62
	9. Pink white.	91.66	18	..	8.34
	10. ,, ,,	95.83	22	..	..
IV White with bluish mottling.	..	97.04	69	23	8.44

TABLE VI. (Contd.)

	1	2	3	4	5
	11 Straw white.	100.00	15	—	8.11
	12 Straw white	87.50	14	—	—
	13 ,, ,,	100.00	15	—	—
	14 Straw brown mottled.	91.66	3	13	8.06
	15 ,, ,,	100.00	8	14	—
	16 Straw ash mottled.	87.50	9	13	8.32
	17 ,, ,,	91.66	8	12	—
	18 Straw brown.	95.80	14	30	9.39
	19 Pink white.	83.33	17	4	8.67
	20 ,, ,,	87.50	20	—	—
x. Brown mottled.	.....	85.50	12	44	7.70
	21 Straw brown mottled.	87.50	1	15	8.17
	22 ,, ,,	100.00	1	19	—
	23. Straw rose mottled.	100.00	11	38	6.42
	24. Straw brown.	100.00	6	37	6.90
	25. Straw white.	77.11	35	—	6.30

Table VI presents the frequency of occurrence of white and pink coloured flowers in the different progeny lines. It was found that almost all lines produced some white flowered plants even if they came from a prental type which had coloured flowers in X_3 . This was the case with lines in type x. Parental types ii and iv also produced some pink coloured plants in some of the lines. Among these lines only the parental lines, 1, 3 of X_3 parental type ii and 11, 12 and 13 of type iv bred true for white flower colour. Other lines disclosed a heterogenity for white and pink coloured flower characteristics by producing both white flowered plants and pink flowered plants within each line.

3. <u>Pollen sterility</u>.

Table VII shows the range of sterility of the parental types, and sterility percentages of five individual plant in each progeny line. It was observed that lines 1, 3, 5 and 6 had not much of variation in sterility (less than 10%) while others had highly variable sterility percentages. Some lines showed plants with values which exceeded the parental range.

In the seed type iv, lines 13, 16 and 18 has sterility percentages which were within the parental range. All others showed greater variability and line 19 had one plant with more than 50% sterility.

TABLE VII.

Comparison of the percentage sterilities in the X_3 parental types with those of X_4 progeny lines.

			Parental sterility range in X_3 39.30% - 20.89%.				
Seed type ii.	Progeny lines.	Plants.	1	2	3	4	5
	1		22.30	23.16	20.91	28.86	28.24
	2		26.53	46.45	21.81	21.17	22.89
	3		26.66	22.70	31.16	25.61	23.33
	4		25.62	37.11	45.57	26.00	37.18
	5		25.80	25.83	27.03	24.27	24.50
	6		27.33	18.24	27.16	23.69	27.41
	7		16.83	22.80	26.96	20.78	30.55
	8		18.33	27.45	48.38	54.15	31.86
	9		27.21	45.73	23.65	17.86	17.80
	10		19.60	29.43	25.66	28.44	32.77

TABLE VII. (Contd.)

Seed type ii.	Progeny lines.	Plants.	1	2	3	4	5
			Parental sterility range in X_3 34.31 - 20.84%.				
	11		38.72	36.78	35.09	28.17	31.30
	12		39.60	28.39	20.55	21.81	23.86
	13		27.10	18.40	20.06	21.87	27.28
	14		24.74	36.00	37.66	32.49	31.35
	15		30.30	30.38	45.24	38.83	27.77
	16		33.08	25.61	35.33	27.39	25.44
	17		39.47	27.27	29.70	20.16	32.04
	18		26.20	32.41	31.96	29.00	29.77
	19		52.22	32.00	47.02	44.53	16.16
	20		31.44	40.88	43.90	27.61	29.07
Seed type x.			Parental sterility range 16.71% to 27.65%.				
	21		36.36	23.91	23.60	21.93	19.00
	22		23.66	22.28	24.12	19.69	22.43
	23		20.65	20.38	19.00	20.88	21.47
	24		25.04	24.80	17.63	32.54	27.65
	25		29.30	20.00	17.18	18.40	18.47

Type x had all lines with sterility percentages which were within the parental range. Also there was not much of variability between lines and between plants in each line.

As a whole, types ii and iv produced marked diaparity in sterility percentages both within lines and within different plants of a line. Inter plant variation was particularly distinct in the seed type iv. Also the highest sterlity (52.22%) was observed in line 19 of this type.

4. Association of Characters.

a. Flower colour with seed colour.

It was observed in the different progeny lines that plants having white flowers had white seed colour also. In the case of the type ii which had pure white seed produced completely white flowers, and type iv which had white seeds with bluish brown mottlings around the eye, produce white flowers with pink shades on the standard and some times partly on the wings. In all but very few cases the number of plants having both white flowers and seeds equalled in frequency. Where there were no white flowered plants, as in lines 21, 22 and 24 there was also the non-occurrence of the white seed type. Therefore it is clear that there is a strong association between these two characteristics.

b. Pink pod colour with pod tip colour.

A direct and close relationship of the two pod characteristics namely pink pod colour and pod-tip colour were observed in four lines (9 and 10 from type ii and 19 and 20 from type iv) which resulted in the occurrence of both characters in equal frequencies.

5. <u>Seed colour type</u>.

Tables VIII (a), VIII (b) and VIII (c) faciliate comparative studies of the different X_4 progeny lined with their X_3 parental types. In the parental type ii, out of five combinations of pod and seed colours studied, none bred true to type but all segregated into different types either with respect to pod colour or seed colour.

A similar case was also observed in the parental type iv, where, out of five lines studied only one line alone bred true and other four lines segregated. (Vide Table VIII (b).

The parental type x was no exception to the general breeding behaviour of the other two types. This also had one progeny line breeding true while others segregated (Vide Table VIII (c).

It was evident that none of the parental types were true breeding. Out of a total of fourteen segregants consisting of 25 lines, only 2 bred true to type. In all cases, the parental types had been predominent among the segregating seed or pod colours.

6. <u>100 Seed weight</u>.

The data collected are presented in Table VI, which

TABLE VIII (a).

Comparative study between Pod and Seed colours of the X_3 parental type with its progeny lines.

X_3 Parental type -'White'

Segregants.

	White.	Brown.	Total.
Straw.	65	2	67
Purple.	42	1	43
Pink.	20	..	20
Total.	127	3	130

Progeny lines in X_4

Lines 1, 2 and 3...... Straw white.

	White.	Brown.	Total.
Straw.	43	2	45

Lines 4 and 5...... Straw Brown.

	Brown.	Ash mottled.	White.	Total.
Straw.	S 31	3	9	43

Lines 6 and 7. Purple white.

	white
Purple	37
Straw	9

Line 8 Purple brown.

	Brown.	White.	Total
Purple.	21	7	28
Straw.	13	..	13
Total.	34	7	41

Line 9 and 10 Pink white.

	White.
Straw	19
Pink shaded.	21
Total.	40

TABLE VIII (b).

Comparison between Pod and seed colours of the X_3 parental type iv with its X_4 progeny lines.

X_3 Parental type IV .... White with bluish mottling around the eye.

	White.	Brown mottled.	Ash mottled.	Brown.	Total.
Straw.	61	11	8	3	83
Pink shaded.	8	1	..	..	9
Total	69	12	8	3	92

Progeny lines.

Lines 11, 12 and 13 - Straw white (Parental)

	White.
Straw.	45

Lines 14 and 15 - Straw Brown mottled.

	Brown mottled.	Brown.	Rose white.	Total.
Straw.	21	7	10	38

Lines 16 and 17. Straw ash mottled.

	Ash-mottled.	Rose white.	Brown.	Total
Straw.	16	17	9	42

Line 18. Straw Brown.

	Brown.	White.	Total
Straw.	30	14	44

Lines 19 & 20 Pink white.

	White.	Brown mottled.	Total
Straw.	5	4	9
Pink	32	..	32
Total	37	4	41

TABLE VIII. (c)

Comparison between Pod and Seed colours of the X_3 parental type X with its X_4 progeny lines.

Parental type X.

X_3 Parental type - Brown mottled.

Segregants.

	Brown mottled.	Rose white.	Brown.	White.	Total.
Straw.	34	7	7	4	52
Pink.	1	1	1	..	3
Purple.	..	..	1	..	1
Total.	35	8	9	4	56

Line 24 Straw Brown.

	Brown.	Rose white.	Total
Straw.	37	6	43

Line 25 Straw white.

	White.
Straw.	35

X_4 Progeny lines.

Lines 21 and 22. Straw Brown mottled (Parental)

	Brown mottled.	Brown.	Total.
Straw.	31	5	36

Line 23. Straw Rose white.

	Rose white.	Brown.	White.	Total
Straw.	29	7	3	39

represent the weights of the parental types and those of the progeny lines. The data show that seed types ii and iv had lines showing more or less equal weights. In seed type x none of the segregants had seed weights which approximately equalled the parental type, but had only lower figures. The straw brown segregants of types ii and iv showed the highest seed weight.

7. Large leaved mutant.

One of the 13 plants isolated in X_3 was carried forward to X_4. 50 seeds were sown and this type showed 87.50% germination. The seedlings from the very beginning revealed large sizes compared to the control. The progenies, were exact replicas of the parental type with their broad dark green leaves and with vigorous growth. The trailing nature of growth, fewer branches per plant and the delayed maturity were characteristic of the parental type. First flower appeared on the 50th day unlike the control type which took only 35 days for the same. There was also considerable variation in flowering among plants. The following studies were made to test whether this is a polyploid.

(a) Pollen size. Measurement of pollen diameter was done for 100 pollen grains under high power (10 x 63) for 10 plants and data compared with those of the control. It was seen that the pollen grains of this type were considerably larger in size to suspect polyploidy.

TABLE IX.

Percentage Sterilities of X_3 parents and X_4 progenies of 'Large-leaved' type.

X_3 Parental value 25.14%

Line 26

Plants.	1	2	3	4	5
Progenies.	30.07	23.45	55.76	35.63	40.49

(b) Leaf thickness and stomatal cells.

These studies revealed that the thickness of leaves and size of stomatal cells of this type were not distinctly different from those of the control.

(c) Meiosis.

It was observed that the number of chromosomes and their behaviour during meiosis were just like those of the control type. The type had formed eleven bivalents at metaphase I and during anaphase I normal separation was observed.

Pollen sterility (Table IX) studies indicated that there was considerable variation from the parental type as well as between plants.

Flower production, pod development, colour of pods and seeds were true to the parental type.

8. Abnormal flower type.

All the pods obtained from the isolated plant in seed type iv of X_3 were carried forward to X_4 generation. The progenies showed good germination like the parental type and were earlier in flower production. Flowers were true breeding in their colour, abnormalities and also in the order of arrangement of floral parts. The types of flower produced by the progenies were studied as follows:-

TABLE X.

Percentage Sterilities of X_3 parents and X_4 progenies of the 'Abnormal Flower' type.

X_3 Parental value 30.84%

Line 27

	Plants.	1	2	3	4	5
Progenies.	3 Standard.	27.83	26.90	26.19	27.94	32.33
	2 Standard.	18.55	26.29	40.57	29.13	23.91
	1 Standard.	33.92	25.53	33.00	23.96	26.67

a. <u>Single standard flowers.</u>

The flowers were either normal like the control with complete floral parts in tact or abnormal with increased or decreased floral parts and these showed irregular arrangements.

b. <u>Double standard flowers.</u>

Here one of the two wings gets modified into the second standard petal. In this case only one wing was found but when two wings were present as in some instance, an increase in floral parts was noticed.

c. <u>Trible standard flowers.</u>

Plants which produced flowers with three standard petals were noted. In addition to the normal one standard, the two wing petals were enlarged and modified to produce the other standards. When present, they caused an increase in the number of corolla parts.

It was interesting to note that all these flower types were triadelphous and tetra adelphous. In many cases the styles were straight unlike the curved styles in the control.

Interplant as well as intra-plant variations in the floral abnormality were noticed in this type. One peculiarity noted was the protrusion of style and stamens earlier to flower opening. Emasculation and crossing of these flowers revealed that the stigmas were receptive.

at the time of emergence.

Table X shows that the percentage of sterility in the parental plant and those of progenies and different flower types do not vary much.

Plates 16, 17 and 18 clearly illustrate the different types of abnormal flowers and their floral parts.

III. Cytological observations.

The behaviour of chromosomes during mitosis and meiosis was studied during the course of the investigation. Root-tip squashes of the X_3 seed material were prepared and studied for all the twenty one seed types. Meiotic studies were also carried out for some of the seed types in X_3 and X_4 particularly for the 'large leaved type'.

1. Mitotic Studies.

It was observed that in all the cases studied, the chromosomes more or less behaved normally in comparison with the control. Normal orientation at metaphase and separation at anaphase were noticed. In no instance any abnormality was observed. Plate No. 19 shows normal metaphase and anaphase stages in root-tip squashes of seed type iv in X_3.

2. Meiotic Studies.

Some good preparations were obtained in the large leaved mutant type. Comparison with the control type was difficult but the various stages obtained revealed a normal behaviour of the chromosomes. Figures 1 and 2 illustrate the various meiotic stages obtained in some of the seed types.

The large leaved type which was suspected to be a polyploid by its gigas characterstics have been proved to be a mutant. This plant, during metaphase showed elevan separate and distinct bivalents (vide firure 3). Synapsis, disjunction, orientation and anaphasic separation were also noticed.

Camera-lucida drawings were made of the stages obtained in mitosis and meiosis and exact figures were prepared from these drawings.

ooo 0 ooo

DISCUSSION

DISCUSSION.

The results obtained in the present investigation on the ' Studies on the morphological and cytological behaviour of X_3 and X_4 generations in cow pea' are discussed and presented below:-

1. Germination.

It was observed by many workers such as Pfiffer and Simmermacher (1915), Jacob (1949) and Spencer and Cabanillas (1956) that X-irradiation hastened germination of seeds. On the other hand Ancel (1924), Kumar and Joshi (1939) and Lesley and Lesley (1957) reported that X-irradiation was deleterious to germination. Still other workers such as Kundu *et al* (1961), Shasthry and Ramiah (1961), Jain *et al* (1961) and Sjodin (1962) reported that germination was independent of X-irradiation. Nair (1964) found germination unaffected when dry seeds were treated while it was considerably reduced when soaked seeds of cow pea (*Vigna sinensis*) were irradiated.

In the present investigation the different seed types in X_3 showed varied germination. Of the twenty one seed types studied, some were early germinating and some were late in comparison to the control. Nine seed types showed higher percentages, four types showed lower percentages and seven seed types showed intermediate germination percentages in comparison to the control.

2. Growth of the seedlings.

The seedlings in X_3 and X_4 generations showed varied growth habit during their development. In final stature, the plants were either erect, spreading or trailing and tall or dwarf. A few large leaved plants were also obtained and one non-branching sterile mutant was found in type xiv in X_3.

These findings were in line with those of previous workers such as Gelin (1954) who obtained multi-branched robust mutants of *Vicia faba* in the X_2 generation and Down and Anderson (1956) who obtained a bushy type of mutant by X-irradiation of the Navy bean which had a spreading habit. Trailing types of *Alopecurus pratensis* was reported by Wohrmann (1956).

Jain *et al* (1962) obtained twelve X_2 progenies in tomato which showed a variable growth habit. Nair (1964) grouped the various X_2 mutants of the 'African' variety of cow pea into twining, profusely branching, dwarf and straight stemmed mutants.

From the foregoing discussion it is evident that various seedling abnormalities and morphological variations are common in the segregating generations of X-irradiated crop plants. The same statement holds good in the present study also in which similar results have been obtained.

3. Sterile Mutants.

Sterile mutants were common in segregating populations of X-irradiated materials. Gustafsson (1947) in barley and Sjodin (1962) in *Vicia faba* obtained such mutants.

Athwal (1963) found that the sterile mutants which he obtained in X-irradiated *Cicer* had luxuriant vegetative growth. He reported two types of sterile mutants. In one, the flowering was normal but the anthers failed to dehisce and the pistil was deformed where as in the other, the flowers were highly abnormal. Nair (1964) isolated two sterile mutants in cow pea of which one plant failed to flower and the other flowered but failed to set fruits.

The results of the present investigation are in agreement with the above reports. One sterile mutant was isolated from seed type xiv in X_3 and three mutants from seed type ii during the X_4 generation. All these plants showed robust vegetative growth and produced flowers but failed to set pods.

Genter and Brown (1941) reported that X-ray treatment caused no significant reduction in pollen sterility in field bean. Tedin and Hagberg (1952) reported a fairly high frequency of partially sterile mutants in X_2 and X_3 generations of Lupin. A number of semisterile plants characterised by high pollen and ovule abortion were observed by Das (1955) in barley. Considerable extent of sterility was noticed by Beachell (1957) during the X_1 to X_3 generations of rice. Similar results have also been observed by Ouang and Chang (1960) and Bozzini (1961).

Jain et al (1961) reported that pollen and seed sterility was not affected much in the X_3 generation of *Chrysanthemum*. The same authors in 1962 reported that X_3 progenies of tomato within a culture showed variation in fertility.

The different seed types showed varying degrees of sterility in the X_3 generation. Highest percentage of sterility was observed in seed type ii. There were types like x, xi and xix which showed a lower degree of sterility than the control. Other types, however, had values more or less equal to that of the control.

Table VII presents a comparison of the percentage sterilities of three X_3 seed types (ii, iv and x) with those of their X_4 progeny lines. Seed type ii which had the highest sterility in X_3 produced some lines which showed lesser extent of variability within themselves and come within the parental range while other lines showed a higher range of variability.

Seed type iv and its progenies showed a similar trend in the variability in sterility. In this case one line in X_4 showed more than 50% sterility unlike the parental type which had only 34% sterility.

However, seed type x which had a sterility range of 16.71% to 27.65% in X_3 produced progeny lines in X_4 with sterility percentage within the parental range.

4. Abnormal Flower type.

Floral abnormalities were observed by Krishnaswamy (1945) in one variety of cow pea (*Vigna ugniculata*). These abnormalities included the occurrence of double standards, wing petal - like growth from the androecium and increase or decrease in the number of floral parts. Breakdown of the papilionaceous structure in the double flowers of *Clitoria ternatea* was observed by Sen and Krishnan (1961). Jain *et al* (1962) isolated plants in X_2 generation of tomato with changes in floral structure consisting of an increase in the number of sepals, petal and stamens. Twiste and divided styles were observed by Jagethesan and Shasthry (1963) in the X_2 generation of *Gossypium hirsutum*. Rana (1964) noted in X-irradiated *Chrysanthemum* a break down of the tubular condition. This was observed only in a mixed tubular type of flowers and appeared quite stable in perfect tubular types.

One plant was isolated in X_3 from seed type iv which produced flowers with an increase or decrease in the number of floral parts, abnormalities in floral parts like the modification of wing petals into normally developed standards, absence or increased number of wing and keel petals, more than one separate single stamen, incomplete development of the staminal column etc., and also abnormalities in the disposition of the various floral parts. This plant was carried forward to X_4 generation and the various abnormalities were found to be true breeding. Sen and Krishnan (1961) suggested that the break down of the papilionaceous structure in the Double flowers of Clitoria indicated a mutation of a single gene. It is assumed that the double type originated through a dominant gene mutation.

One interesting peculiarity noticed in the X_4 generation was that in the latter part of the flowering season almost normal flowers were found on these abnormal plants.

Another feature peculiar to this abnormal type was that the inter-plant variation with respect to floral abnormalities. Intra-plant variation was also observed by Rana (1964) and he is of opinion that such a situation can best be explained by assuming the gene determining the tubular condition to be unstable. Such a locus mutates so frequently in the course of flower development in a plant that a highly variable phenotype is obtained. The intra - plant variation observed in the present investigation could similarly be explained.

However, the inter - plant variation for differential expressivity of the tubular character is difficult to explain according to the above interpretation. Other possibility according to Rana (1964) is that the expressivity of the gene responsible for the mutant condition is variable depending upon the genotypic back ground of the plant.

Incomplete and variable expression of certain mutant types was noted by early investigators (Timoffeeff-Ressovsky 1927, and Morgan 1929). It is becoming clear now that the variable expressivity of genetic potentialities is conditioned by modifier systems (Goldschmidt 1938, Bezem and Sobels 1953 and others). If a certain gene has variable expressivity, apparently its action during development is weak and can be modified by the action of other genes and also by external factors.

These explanations envisage a possible interpretation of the inter-plant differential expressivity observed in the abnormal flower type. It may be believed that modifier gene systems interact with the genotype of the plant and cause variability in expression.

Rana (1964) opined that qualitative variation may assume a pattern of the quantitative type through variable expressivity of typical Mendelian genes. Hence a proper consideration of variable expressivity of genetic potentialities will prove useful towards better understanding of the quantitative type of inheritance.

5. Chlorophyll Mutants.

Chlorophyll variations noticed in the X_3 generation included partial absence of chlorophyll, chlorophyll deficient spots unevenly distributed yellow patches and xantha mutants. These were mainly noted in seedlings. Chlorophyll variegations were also observed in a few seed types in the later stages of the plant growth.

Baburao and Kadam (1941) analysed the different deficiencies and grouped the various types as albino, lethal yellow, stunted yellow, twisted-pale green and tipburn yellow in X-irradiated rice. Gustafsson (1940) classified different chlorophyll mutants and viable chlorophyll mutants in barley. The former included xantha, albino, virescens and chlorina while the latter group includes mutants like albo-viridis, viridis and tigrina.

Blixt (1961) identified a type of chlorophyll variegation in X_2 and X_3 of peas which was characterised by patches of green and yellow spots and he named it as 'chlorotica vario maculata'. During the course of X_3 in the present study, four xantha seedlings were obtained and seedlings with yellow and green spots were observed in a total of twelve.

Sjodin (1962) reported that chlorophyll mutations were comparatively rare in the segregating generations of leguminous plants. This is in agreement with the results obtained in the present studies that in a large population of X_3 plants only a few chlorophyll mutations were observed. Nair (1964) recorded a total of nine albinos and seven xanthas in the X_2 generation of cow pea and a number of chlorophyll spotting on the first leaves of few seedlings. Similar types were also observed in X_3 in the present study.

6. Large-leaved Mutant.

Gustafsson (1947) working with barley observed broader and narrower leaved mutants in the X_2 and X_3 generations. Some gignatic plants were observed by Jacob (1949) in X-rayed *Corchorus* plants.

Seed types xiv and XV in X_3 produced thirteen 'large leaved' plants which were characterised by large, dark green leaves, with trailing growth habit and shy branching nature. They were found to be late in flowering but produced larger pods.

The plants in general showed gigas characteristic and bred true in the X_4 generation. Cytological studies revealed that this is the result of gene mutation and not due to any detectable chromosomal aberrations. Similar mutants were reported by Nair (1964) in the X_1 generation of X-irradiated cow pea.

7. Flower colour types.

Flower colour changes were recorded as early as 1930 when Goodspeed observed them in the progenies of irradiated tobacco and also by various workers like Bruns (1954) Hoffmann, Zoschke (1955), and others.

Rai and Jacob (1956) isolated a white flower mutant in X_3 generation of <u>Sesamum</u> and a similar mutant in mustard. Nair (1964) observed flower colour changes in the X_2 generation of cow pea. The colours ranged from deep pink to complete white with various intermediate shades.

Similar results have been obtained in the present studies in X_3 and X_4 mutant cow pea which showed flower colour changes from deep pink to complete white. Nine new types were observed in X_3. Flower colour changes were frequent in the different seed types studied in both X_3 and X_4 generations. It was found that most of the seed types produced some white flowered plants in the progeny. This indicates that the seed types were not pure for flower colour character.

8. <u>Seed colour Mutants</u>.

The most interesting and important character studied in the present investigation was the seed colour of mutant types. Similar seed colour mutants were observed and recorded by different workers as early as 1931 when Stadler observed a large number of seed colour mutants in irradiated <u>Zea mays</u>. Interesting seed coat colour variations were reported by other workers as Nishimura and Kurakami (1952) in rice, Nair (1961) in <u>Sesamum</u>, Sjodin (1962) in <u>Vicia faba</u>. Nair (1964) obtained twenty one mutant seed types from the X_2 generation of cow pea which formed the basis of the present investigation. The different colour changes included "whole colour" types such as white, light-red and brown, "eye colour" changes like white seeds with black eye, and "mottlings" like dark mottling, violet mottling etc.

Out of the twenty-one seed types studied in X_3 it was found that a total of eight X_2 seed types bred true to type, (four with respect to seed colour and pod colour and three for seed colour alone and one for pod colour alone). All the remaining types segregated with respect to their pod and seed colours. Almost all X_2 seed type colours were observed in the X_3 population. Among the segregating lines of the three X_3 parental types, lines 1, 2, 3, 6, 7, 9, 10, 11, 12, 13, 19, 20 and 25 bred true while other lines showed segregation for different seed coat colours.

In *Vigna*, four factors were reported to be responsible for the ' whole colour' by Harland (1920, 1922). They were 'B' 'N' 'M' and 'R' and the various combinations gave various colours. 'R' forms the basic gene in the absence of which the seed colour would be white.

Spillman and Sando (1930) suggested eight factors for seed coat colour in *Vigna*. They are 'B' (Brown), 'R' (Red), 'U' (White) 'P' (Purple) 'F' (dense bluish mottled) 'T' (sparse mottled) and 'X' (inhibitor gene which inhibits the effect of 'F'). Various combinations of these eight genes gave different seed coat colours. Any deletion or mutation would lead to seed colour changes.

Based on this explanation, the different seed coat colour development in the present investigation could be discussed.

9. Cytological abnormalities.

Cytological analysis of mutants from X-irradiated Nicotiana sylvestris and N. tabacum by Fujii (1955) revealed chromosomal aberrations including translocations, univalents, and asynaptic configurations. Onnfrijchuk's (1953) cytological studies of one speltoid mutant in barley in X_3 showed that this mutant had 21 bivalents + 1 isochromosome. It is assumed that in the production of this X_3 mutant at least two reciprocal translocations would have occurred. Korah (1958, 1959) reported a number of meiotic and mitoitic aberrations in X-irradiated Oryza sativa. Shasthry and Ramiah (1961) observed in the X_2 that multivalents were the most common abnormality in the M_2 generation of irradiated rice.

Contradictory results have been reported by Parthasarathy (1939) from the cytological analysis of X_3 semisterile rice mutants. He observed that these mutants contained the normal complement of chromosomes and the meiosis was regular. He suggested that the origin of the mutants may probably be through a small deficiency or a gene alteration. Similar findings were obtained by Athwal (1963) who studied the various X-irradiated and spontaneous mutants during their X_2 generation to detect chromosomal aberrations. No meiotic irregulairty was seen in any of these mutants.

Cytological studies on X_3 and X_4 generations in cow pea fully agree with the second set of observations. Out of the twenty one mutant seed types studied either by smears of PMC or by root tip squashes, no chromosomal abnormality was observed. The 'large leaved' mutant and 'abnormal flower' types also showed normal meiosis and 11 bivalents were observed in PMC smears. It may therefore be assumed that these two mutant types may be the result of some undetectable chromosomal aberrations or gene mutations.

:- oo 0 oo : -

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

This piece of work embodies the results of an investigation carried out to study the morphological and cytological behaviour of mutant cow pea in the X_3 and X_4 generations. Twenty one seed types obtained from X_2 formed the basis of the X_3 generation. Three selected seed types from X_3 were raised in the X_4 generation. Various studies on the morphological characters supplemented with cytological studies were conducted during both generations.

Germination, chlorophyll variations, growth habit of plants, sterile mutants, flower colour types, seed colour mutants and some other mutants like abnormal flower type and 'large leaved' mutant type were studied. In addition to this, cytological studies to understand the behaviour of these mutant types during mitosis and meiosis were undertaken. No cytological abnormality was recorded in any of the types studied.

It was observed that the different seed types showed varied germination percentages. Higher, intermediate and lower values were obtained in both X_3 and X_4 generations. Chlorophyll variations were noticed only in a few seed types and were comparatively rare in the X_4 generation. Abnormalities in the number of leaflets were also observed in some of the seed types in X_3.

Plants were found to have varying growth habits as erect, spreading, trailing or twining tall or dwarf and branching or nonbranching in both generations. Nine new flower colour types were obtained in the X_3 generation.

Pod colour types obtained were studied during X_3 and X_4 generations. Also the breeding behaviour of the different seed colour types were studied in combination with their pod colours. Different seed colours recorded were grouped as parental and segregating types in both the generations.

Pollen sterility in different seed types showed variations in X_3 and X_4 generations.

Four pure breeding progeny lines with respect to both pod colour and seed colour were isolated from the X_4 generation. Three lines which bred true for seed colour alone and one which was true breeding with respect to pod colour were selected. Thus altogether eight lines were selected for further studies.

The important results obtained in the X_3 and X_4 generations were discussed in detail.

Scope and value of the present Investigation.

Mutation breeding has two objectives i.e., (1) to increase genetic variability and (2) to produce a specific mutation that will confer a desired character to an otherwise superior variety (Myers 1960).

It may be presumed that the first condition was achieved in cow pea by X-irradiation which resulted in the selection of twenty one different seed types from the X_2 generation. The present investigation provided ample chance for the various mutant types to express their breeding behaviour with respect to the genotypes. Pure breeding genotypic lines were selected from the X_4 generation for further studies. Further investigation with these pure breeding lines to select better yielding strains is suggested.

--ooOoo--

REFERENCES

R E F E R E N C E S.

Abrams., and Velez - Fortuno, J. 1962. Radiation research with pigeon-peas (cajanus cajan): Results on X_3 and X_4 generations. J. Agric. Univ. P.R. 46: 34-42.

Ancel, S. 1924 Action de faibles doses de rayons X sur des grains seches Compt. Redn. Soc. Biol. (Paris) 91: 1453 - 1436 (cited by Johnson, E.L. In, biological effects of radiation, Vol.II (Ed) Duggar Pp.961-985 Mc - Graw - Hill Book Company, Inc. New York.

Anonymous. 1955 Report of the Bose Institute - Calcutta for 1953 - '54 Pp.100., and for 1954 - '55 Pp. 124.

.... 1958 Annual report of the Indian Central Jute Committee for the year 1955 - '56. PBA. 28: No.3 Pp 292.

Athwal, D.S. 1963 Some X-ray induced and spontaneous mutations in Cicer. Indian J. Genet. 23. 50-57.

Baburao, and Kadam, S. 1941 Genetic analysis of Rice. ii-Chlorophyll deficiencies. Indian J. Genet. 2: No.4

Beachell, M.M. 1957 The use of X-rayed thermal neutrons in producing mutations in rice. Rice Comm. 6: 18-22.

Bhatia. and Swaminathan, M.S. 1963 An induced multiple carpel mutation in bread wheat. Genetica. 34: 58-65.

Blixt. 1961 Quantitative studies of induced mutations in peas: V. Chlorophyll mutations. Agri. Hort. Gene. 19: 402-447.

Bora, K.C. and Rao, N.S.	1960	Experiments with rice (Oryza - sativa) on the induction of mutation by ionizing radiations. Isotopes in Agriculture. U.N. procc. on the peaceful uses of Atomic Energy. 1958. 306-313.
Bozzini, A.	1961	Chromosome mutants induced by ionizing radiations in the hard wheat Capelli. Alli. Assoc. Genet.ital.6: 365-370.
Bruns, A.	1954	The induction of mutations through X-irradiation of dormant seeds of Trifolium Pratense. Angew. Bot. 28: 120 - 55.
Campos, F.F., and Espiritu, L.	1966	Mutants isolated from X_1 and X_2 generations of rice (Oryza-sativa). Phillip. Agric.44: 299-307.
Carpenter, J.A.	1958	The induction of mutation in subberranean Clover by X-irradiation. J. Aust. Inst. Agric. Sci.24: 39-44.
Chaudheri, K.L.	1963	Early maturing X-ray mutations of Jute, (Corchorus olitorius. Linn). Trans. Bose Res. Inst.19: 89-105.
Chaudhuri, K.L. and Das, K.	1956	Effect of X-rays on the fertility of pollen grains in Sesamum orientale. Sci. and cul.21: 550-553.
Das, K.	1955	Cytogenetic studies of partial sterility in X-ray irradiated barley. Indian J. Genet. 15:
Down, E.I., and Anderson, A.L.	1956	Agronomic use of an X-ray induced mutant. Science. 124: 223.24.

Ehrenberg, A. Gustafsson, A, and Lundquist, U. 1961 Viable mutants in barley by ionizing radiations and chemical mutagens. Hereditas. 47: 243-283.

Elliot F.C. 1955 Spring wheat breeding and the transfer of economic characters from related species and genera. Wheat. Inform. Serv.Kyoto: 3:30

Freislben, R and Lein, A. 1943 Preliminary work on the breeding result of X-ray induced mutations. I. Visible action of irradiation in treated generation (X_1) of dormant barley seeds. Z. Pflanzenzuchtt. 25: 235-254.

Froier, K. 1946 Genetic studies on the chlorophyll apparatus on Oats and Wheat. Hereditas. 32: 297-406.

Fujii, T. 1955 Mutations in Einkorn wheat induced by X-rays., I. Chlorina mutants. Proc. Japan. Acad: 31: 88-92.

Gelin, O.E.V. 1954 X-ray mutants in Peas and Vetches. In Mutation Research in palnts. Acta - Agric. scand. 4: 558-68.

Genter, and Brown. H.M 1941 X-ray studies on the field bean. J. Hered. 32: 39-44.

Gladstones, J.S. 1958 Induction of mutation in the West Australian blue Lupin (Lupinus digitatus., Forks) by X-irradiation. Aust. J. Agric. Res. 9:473-82.

Goodspeed, T.H. 1928 The effects ofX-rays and redium on spacies of the genus. Nicotina. J. Heredity 20.

Gottschalk, W.	1958	Genetic problems of mutation breeding in Peas (Pisum Sativum). In "Effects of Ionizing Radiation on seeds". Reference cited by Gotteschalk (1960) I.A.E.A. symposiun, Vienna, 1961.
Gottschalk, W. and Scheibe.	1960	Genetic problems of mutation breeding in peas (Pisum sativum). In "Effect of ionizing radiations, on seeds. I.A.E.A. sympossium. Vienna Pp. 465-471.
Gregory, W.C.	1955	X-ray breeding of pea nuts. (Archis hypogaea.L). Agron. J. 47: 396-399.
....	1956	Induction of useful mutation in Pea nut. In ' Genetics in plant Breeding'- Brook haven Symposia in Biology No.9, Pp.177-191.
Gregory, W.C., and Cooper, W.E.	1959	"Atomic Peanut". Res. and Fmg.17: No.4 Pp.3.
Gustafsson, A.	1940	The mutation system of Chlorophyll apparatus. Acta. Univ. Lund. 236: 1-40.
....	1947	Mutations in Agricultural plants. Hereditas. 33: 1-100.
Hackbarth, J.	1955	Experiments on the induction of mutations in Lupinus luteus.L. angustifolins and L.Angustifolius and L. albus by X-irradiation. Z. Pflanzenz. 34: 375-90.
Harland, S.C.	1920 1922	Reference cited by Krishnaswamy et al (1945). " Studies in cow pea Madras. Agric. Jour. No. 8.1945.
Hoffmann, W., and Killough, D.T. Zochke, U.	1955	X-ray mutations in Flax. (Linum usitatissimum) Zuchtes. 25: 199-206.
Horlacher, W.R., and Killough, D.T.	1931 1932	Radiation induced variation in Cotton. Somatic changes induced in X-raying seeds. J. Heredity. 22: 253-262.

Jacob, K.T.	1949	X-ray studies in Jute-II. A comparative study of the germination percentage, size and external morphology with different doses of X-rays. Transactions of the Bose.Rs.Insti. Calcutta Vol.xxviii. 23-29.
Jagathesan, D. Swaminatha, M.S., and Puri, R.P.	1963	Breeding for resistance to Jassids in Cotton - use of induced mutations. Indian Cot. Gr. Review. 17: 96-99.
Jain, H.K. Bose, A.K. SathpathyD and Sur, S.C.	1961	Mutation studies in annual Chrysanthemum. 1. Radiation induced variation in flower form. Indian J. Genet. 21: 68-74.
Jain, H.K Sur, S.C., and Rant, R.N.	1962	Genetic studies in Tomato. 1 Induced variability for uniform fruit ripening and other characters. Indian. J. Genet. 22: 81
Katayama, T.	1963	X-ray induced Chromosomal aberrations in rice plants. Japanese. J. Genet. 38: 21-31.
Kato, K.N., Hu, C-H, Chang, W-T., and Oka, H-I.	1960	A biometrical genetic study of irradiated populations in rice, genetic variances due to different doses of X-rays. Bot. Bull. Acad. Sin; 1: 101-108.
Korah, M.	1958	Two rare chromosomal abnormalities in Oryza sativa L induced by X-rays Pyton 11, (2) 12: 97-101.
....	1959	Cytotaxonomical, radiation and cytological studies in Oryzasativa. I. Effect of gases on the mutagenic capacity of X-rays. Ph.D Thesis, Calcutta University. (unpublished)
Krishnaswamy, N. Nambiar, K.K., and Mariakulandi, A.	1945	Studies in cow pea (Vigna ugniculat L. Walp). Madras Agric. Jour.No. 7 Pp.145-161 No. 8. Pp.193-200.

Kundu, B.C., Ghosh, K., and Sharma. 1961 Studies on the effect of X-irradiation in Corchorus capsularis. L. and C. Olitorius. Genetica 32: 51-73.

Kumar, L.S.S and Hoshi, W.V. 1939 Experiments on the effect of X-rays on Pennisetum typhoides Nicotiana tabacum, and Brassica juncea. Indian J. Agric. Sci. 9: (4). 675-684.

Lamprecht, H. 1958 On basic genes for the formation of higher plants and on new and already known X-ray mutants". Agri.Horti.Genet.16: 145-195.

Lesley, J.W. and Lesley, M.M. 1956 Effect of seed treatments with X-rays and P^{32} on tomato plants on first, second and third generations. Genetics. 41: 575-588.

Levan, A. 1944 Experimentally induced chlorophyll mutants in Flax. Hereditas.30: 225-230.

Maldiney, and Thouvenin. 1898 Reference cited by E.L.Johnson In Biological effects of irradiation. Vol. II.Duggar, B.M.(Ed.) McGraw-Hill Book Company, Inc. New York.

Marki, A., Sebok, C. and Rusu, E. 1962 Contributions to the study of the third generation of soybean irradiated with X-rays. Stud.Cercet.Biol.Cluj.13: 161-165.

Mashimo, I. and Sato, H. 1959 X-ray induced mutations in Sweet Potato Jap.J.Breeding. 8: 233-237.

Matsumura, S. and Fujii, T. 1955 Gene mutation in Einkorn wheat induced by X-rays. Wheat Inform.Serv.Kyoto.No.2 13-40.

.... 1955 "Studies on tobacco mutants induced by X-ray irradiation. Jap.J.Breeding. 5: 41-46.

Murray, B.E. and Craig, I.L.	1962	A cytogenetic study of the X-ray induced cauliflower head and single leaf mutation in *Medicago sativa*. *Canad.J.Genet.Cyt.4*: 379-385.
Myers, W.M.	1960	Some limitations of Radiation Genetics and Plant Breeding. *Indian J.Genet.20*: 89-92.
Nair, G.G.	1961	Small seeds--X-rays induced higher yielding mutant in *Sesamum orientale* L. *Sci.& Cul. 27*: 310-311.
Nair, P.N.R.	1964	Investigations on the effects of X-rays on cow pea (*Vigna sinensis* L. SAVI.) M.Sc. Thesis (unpublished), Kerala University.
Narahari, P.,and BORA, K.C.	1963	Radiation induced spikelet abnormalities and mutations in rice *Indian J.Genet.23*: 7-18.
Nirad Sen, and Krishnan.	1961	Break down of the Papilionaceous Structure in the Double flowers of *Clitoria ternatea* and its inheritance. *Curr.Sci. 30*: 435.
Nishimura, Y. and Kurakami, H.	1952	Mutations in rice induced by X-irradiation. *Japan.J.Breeding 2*: 65-71.
Oka, H-I., Hayashi, J. and Shiojiri,I.	1958	Induced mutation of poly genes for quantitative characters in rice. *J.Hered.49*: 11-14.
Ouang, T.Y. and Chang, M.T.	1959	Mutations in rice induced by X-irradiation. Progress in Nuclear energy. Series VI. *Biological sciences. 2*: 22-28.
Onnfrijchuk,T.	1953	Production of speltoid mutants in spring wheat by X-ray irradiation. Plt.breed.Abst.2547: *23*: Pp.553.

Palenzona, D.L.	1962	Consequences of treatment of seeds with X-rays in Triticum aestivum. Atti.Assoc.Genet.Ital. 8: 314-321
Papa, K.E. and Williams, J.H.	1959	Selection for quantitative characters in the 3rd generation following irradication of soybean seeds with X-rays and thermal neutrons. In "Abstracts of the Annual meeting of the American Society of Agronomy Ohio, 1959.
Parthasarathy, N.	1938	"Cytogenetical Studies in Oryza and Phalardieae. I-Cytogenetics of some X-ray derivatives in Rice (Oryza sativa)" Jour.Genet.37: 1-40.
Patil, S.H. and Bora, K.C.	1963	Radiation induced mutation in groundnut. I-Chlorophyll mutations Indian J.Genet.23: 47-49.
Pfieffer, T. and Simmermacher, W.	1915	The influence of Roentgen rays on the seeds of Vicia faba as shown in the development of the plants. Landw.Vers.Stat.86: 35-43 (Cited by Johnson, E.L. in biological effects of radiation (Ed) Duggar, B.M. 1936 McGraw Hill Book Company Inc.New York.
Rai, U.K.	1956	X-ray induced appressed pod mutant in Brassica juncea Sci. & Cul. 24: 46-47.
....	1959	X-ray induced high yielding early flowering mutants in mustard (Brassica juncea) Genetica. 30: 123-128.
....	1959	Thickened pod--a morphological recessive mutant in X-ray treated Brassica juncea. Sci.& Cut.24: 534.
Rai, U.K. and Jacob, K.T.	1956	Induced mutation studies in Sesamum and mustard. Sci.& Cul.22: 344-46
Rana, R.S.	1964	Phenotypic variability of an induced mutant of annual Chrysanthemum genetica. 35:236-240

Sears, E.R.	1956	The transfer of Leaf-rust resistance from Aegilops umbellulata to wheat (Triticum vulgare) In 'Genetics in Plant Breeding. Brook haven symposia in Biology No.9: Pp.1-23.
Sen, N.K. and Bhowal, J.G.	1962	"A male sterile mutant cow pea" J.Hered. 53: 44-46.
Shastry, S.V.S. and Nadhachary.	1965	X-ray induced mutations in cultivated Rice-NP. 130. Curr.Sci. 34: 55-56.
Sikka, S.M., Swaminathan, M.S. and Jagathesan, D.	1956	A note on some X-ray induced variations in upland cotton. Indian J.Genet.16: 144-145.
Sjodin, J.	1962	Some observations in X_1 and X_2 generations of Vicia faba after treatment with different mutagens. Hereditas: 565-586.
Spencer, J.L. and Cabanillas.E.	1956	The effect of X-rays and thermal neutrons on the development of trailing Indigo (Indigofera endecaphylla) plants. Am.J.Bot.43: 289-296.
Stadler, L.J.	1928	Mutations in barley induced by X-rays and radium. Science 68: 186-187.
....	1932	On the genetic nature of induced mutations in plants. Proc.Sixth Int.Cong.Genet.1: 274-294.
Tan, J. and Hehn, E.R.	1961	Cytological investigations of irradiated wheat x rye derivatives".
Tedin, O. and Hagberg, A.	1952	Studies on X-ray induced mutation in Lupinus luteus L. Hered.Lund: 38: 267-96.
Vettel, F.K.	1959	Mutation experiments on wheat-rye hybrids (Triticale) I-Induction of mutation in Triticale, Rimpan. Zucher.29: 293-317.

Wohrmann, K.	1955	Investigations on the Physiology of germination, fertility and the cytology of progenies from X-irradiated seeds of *Alopecurus pratensis.*
Yagu, P. and Morris, R.	1957	Cytogenetic effects of X-rays and thermal neutrons on dormant tomato seeds. *Genetics* 42: 222-238.
Yaguchi, H.	1959	On the estimation of segregation of chlorophyll mutants in the progeny of irradiated rice. *Jap.J.Breeding* 9: 128-134.
Yamaguchi.	1959	On the expressivity of an awn character in barley induced by X-rays. Ikushugaku Zasshi *Jap.J.Breeding* 9: 28-32.
Zacharias, M.	1956	Mutation experiments on crop plants, VI. X-irradiation of the Soybean (*Glysine soja* L.) *Zuchter* 32: 1-38.
....	1956	Reference cited by Sjodin, J. (1962). (*Hereditas.* 48: 565-586)
Zachow, F.	1958	The inheritance and discovery of some X-ray induced mutations of *Lupinus luteus.* *Zuchter.* 28: 262-268.
Zwintzscher, M.	1955	The production of mutants as a method of fruit breeding.I. The isolation of mutants with reference to primary changes. *Zuchter.* 25: 290-302.

==:xox:==

PLATES

Fig. 1 Camera lucida drawings of Anaphase I in seed type ii.

Photomicrograph of the PMC showing Anaphase I.

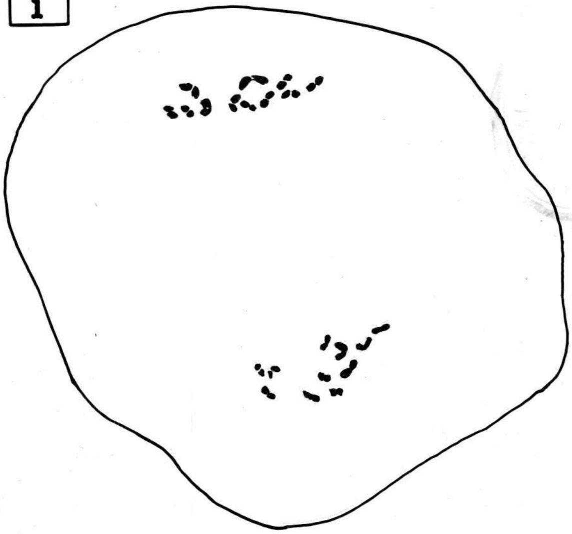
Fig. 2 Photomicrograph of the PMC in Metaphase I showing 11 bivalents, from the Large mutant.

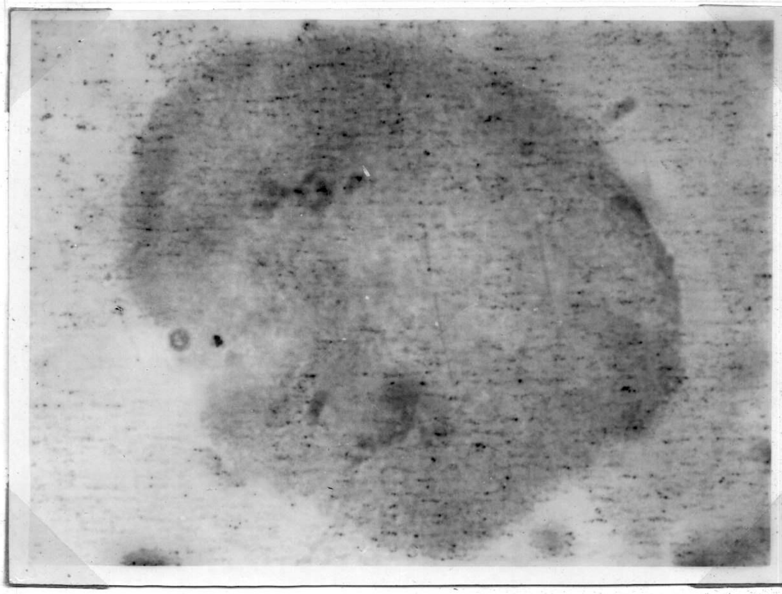
Camera lucida drawings of the corresponding stage at Metaphase I.

Fig. 3 Camera lucida drawings of Metaphase I from seed type iv.

Photomicrograph of Metaphase I.

FIG 1 SEED TYPE ii ANAPHASE I





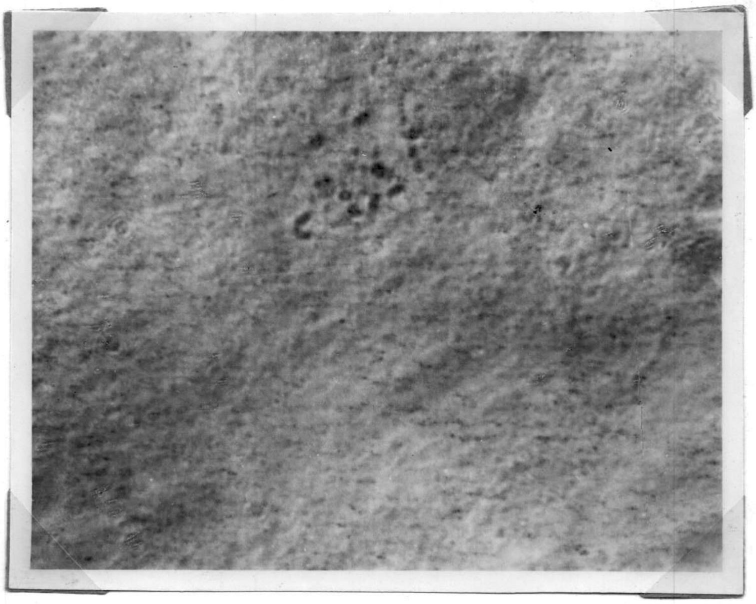


FIG 2 LM METAPHASE I

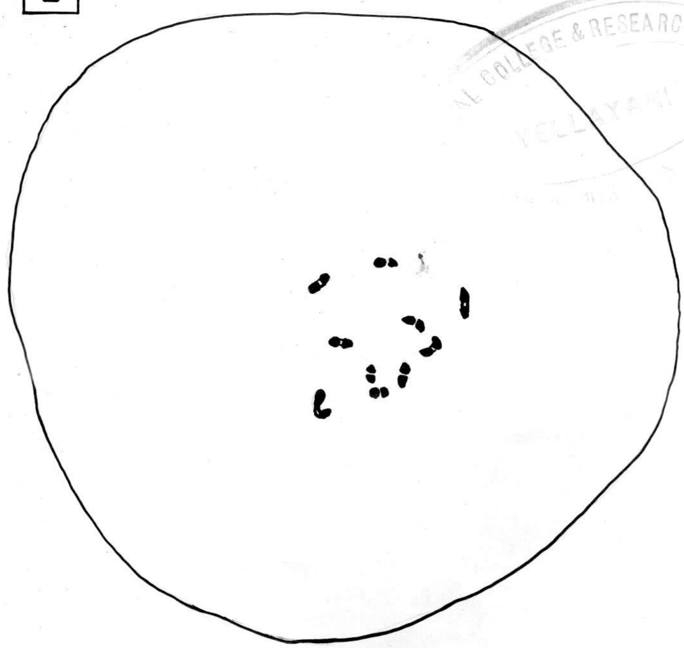


FIG 3 SEED TYPE iv METAPHASE I

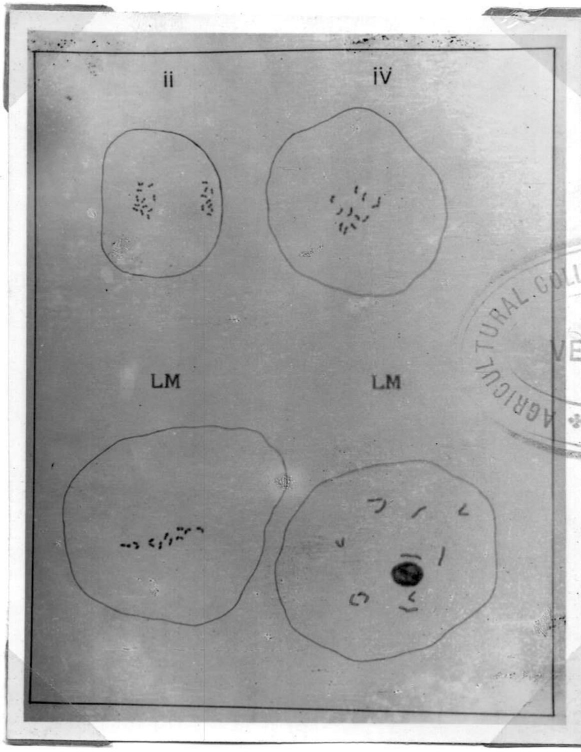
Plate showing normal meiotic stages in some of the seed types. (Camera-lucida drawings)

ii. Anaphase I observed in seed type ii.

iv. Metaphase I observed in seed type iv.

LM. Metaphase I and Diakinesis observed in the PMC of Large leaved mutants.

Plate showing normal somatic Metaphase and Anaphase in seed type iv.


ii
iV
LM
LM

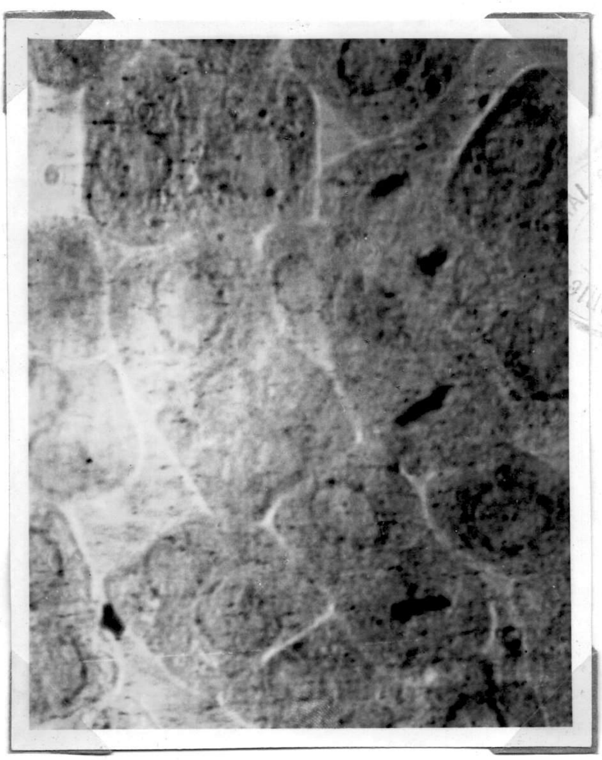


PLATE 1. General view of the Field Trial.

PLATE 2. A normal Cow pea plant of the variety 'African'.

PLATE 1

PLATE 2

PLATE 4.	xiii.	Large brown.
	xiv.	Small brown.
	xv.	Brown - medium sized.
	xvi.	Reddish white.
	xvii.	Half - red half - white.
	xviii.	Deep violet mottled.
	xix.	Deep ash mottled.
	xx.	Greyish mottled large seeds.
	xxi.	Brown mottled.
	xxii.	Light yellowish small seeds.

Seed material used in X_3 generation.

Plate 3	i.	--- 'African' variety of Cow Pea - Control.
	ii.	--- White seed type.
	iii.	--- White with brown patch around the eye.
	iv.	--- White with bluish mottlings around the eye.
	v.	--- White with red patches around the eye.
	vi.	--- White with black patch around the eye.
	vii.	--- Reddish with black mottlings.
	viii.	--- Deep brown mottled.
	ix.	--- Light brown mottled.
	x.	--- Reddish white and brown mottled.
	xi.	--- Yellowish white with light brown mottlings.
	xii.	--- Grey - ash mottled.

.....

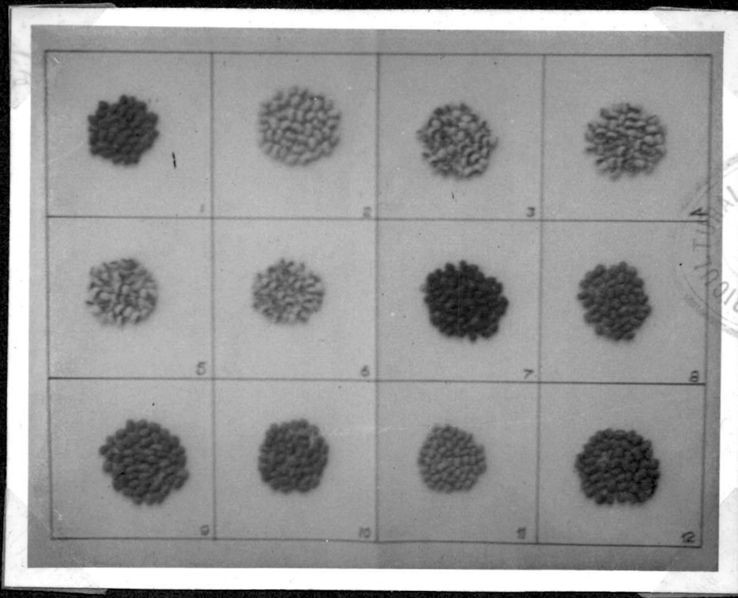


PLATE 3

PLATE 4

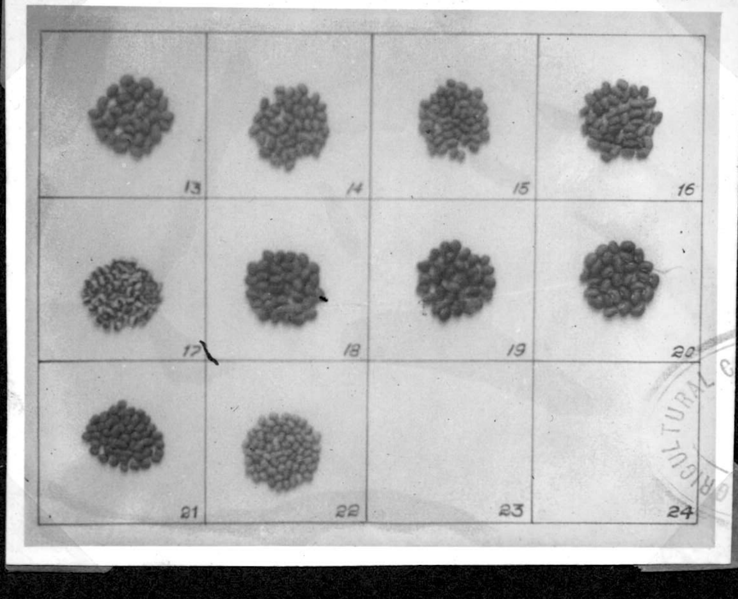


PLATE 5. Leaflet abnormalities observed in the X_3 generation.

PLATE 6. Chlorophyll variations noted in seed types ii and xix in X_3.

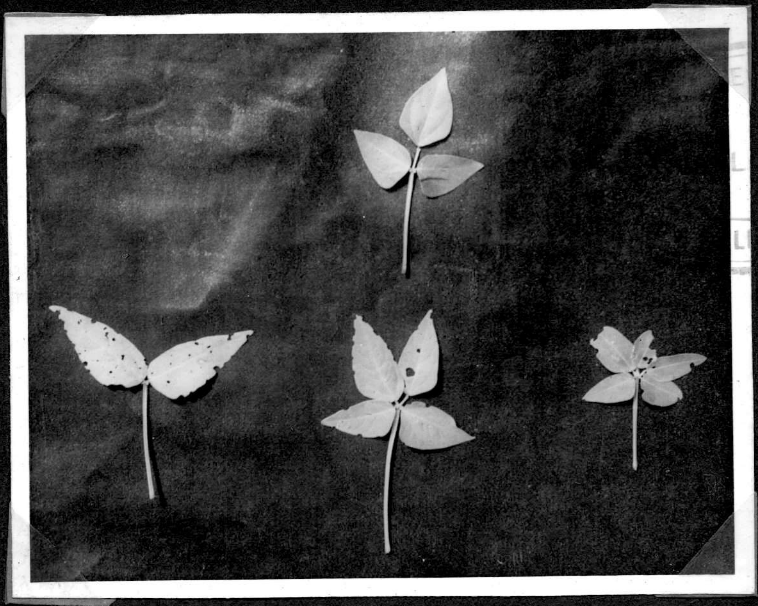


PLATE 5

PLATE 6

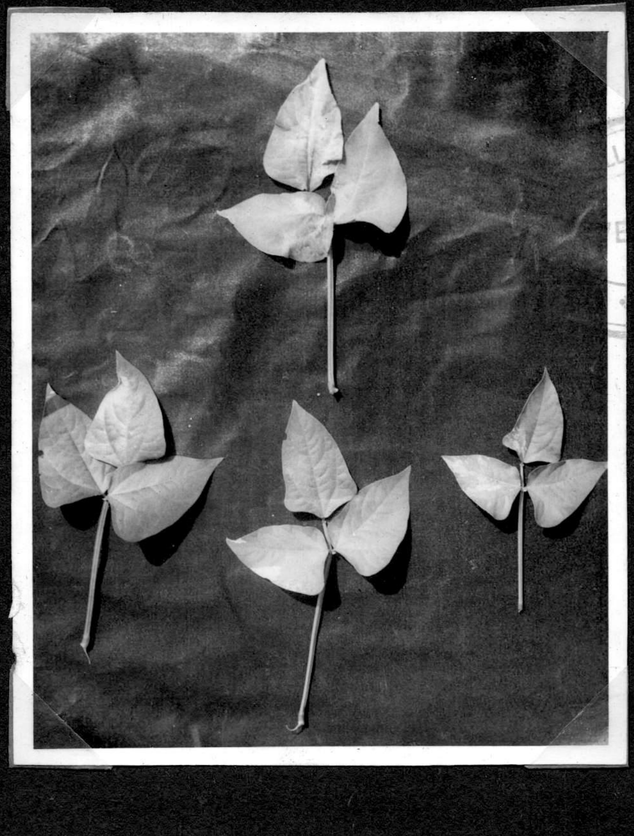


PLATE 7. One dwarf mutant in seed type iv in X_3.

PLATE 8. A large leaved (LM) from seed type xv in the X_3 generation.

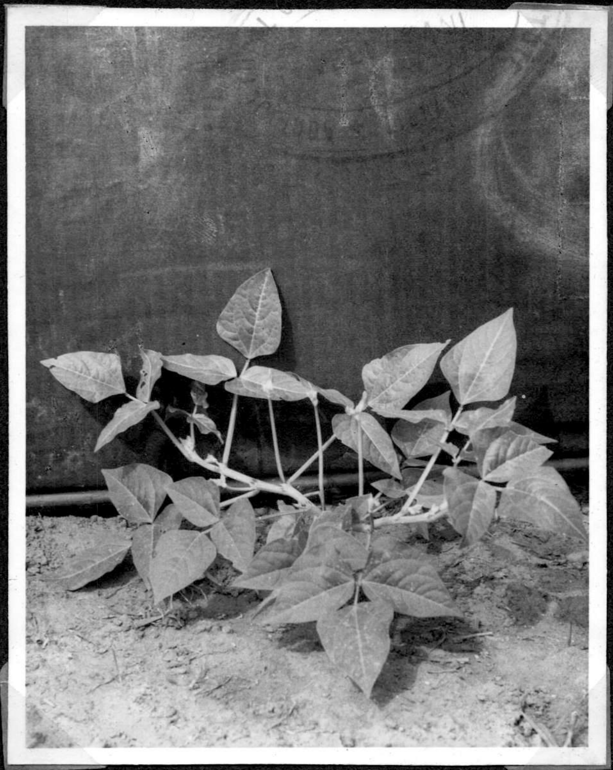


PLATE 7

PLATE 8

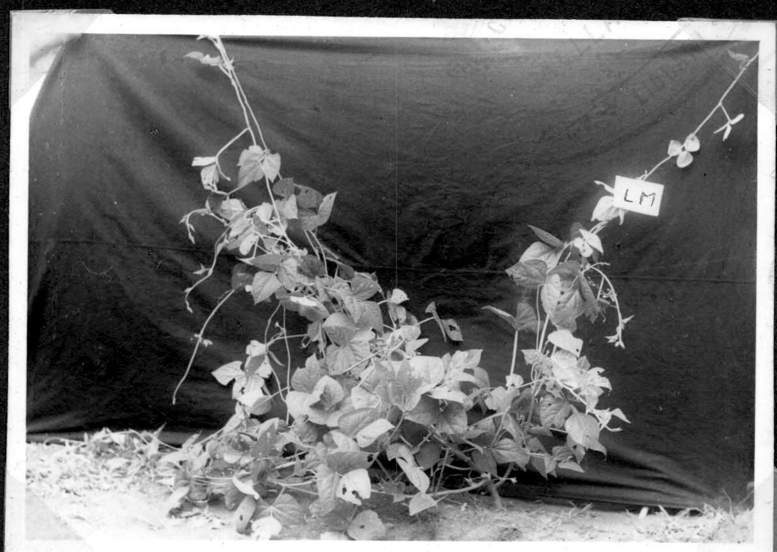


PLATE 9. A non branching Sterile mutant obtained from seed type xiv in X_3.

PLATE 10. A spreading mutant with large pods from seed type xviii in the X_3 generation.

PLATE 9

PLATE 10

Pod types obtained in X_3

PLATE 12.

1. Small light yellow pods.
2. Medium straw pods (control).
3. Medium broad straw pods.
4. Medium light yellow pods.
5. Broad and round straw pods.
6. Large broad light straw pods.
7. Large light pink shaded pods., and
8. Large broad round light yellow pods.

PLATE 13.

9. Large broad pink shaded pods.
10. Long pink- variegated pods.
11. Medium pink shaded pods.
12. Medium purple - tip coloured pods.
13. Medium purple pods, and
14. Medium light purple pods.

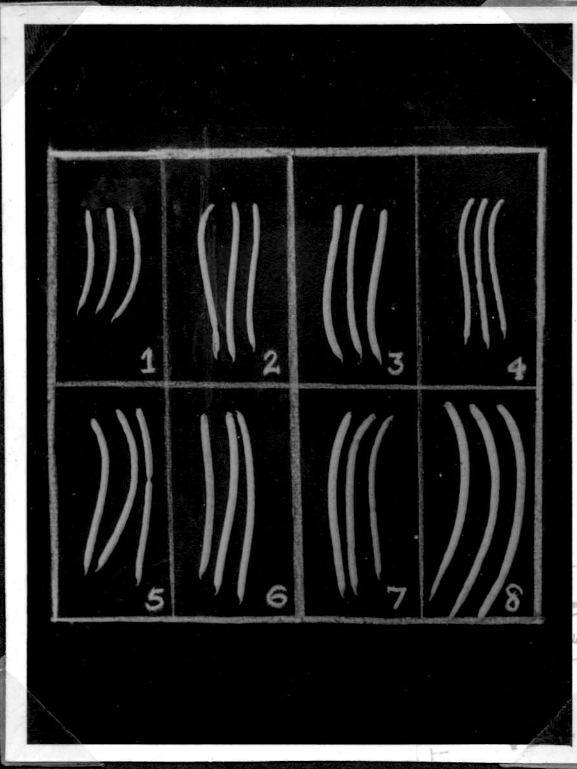
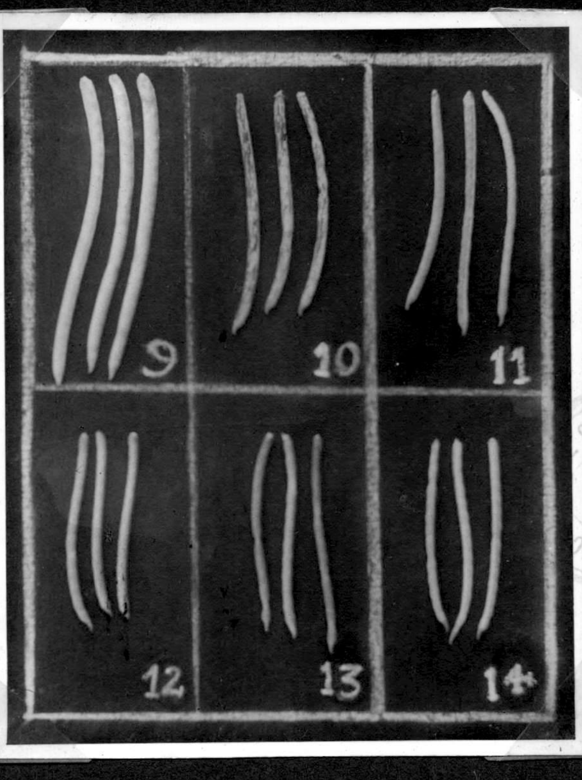


PLATE 11

PLATE 12



X_4 Seed material.

PLATE	13.	A.	1.	Straw White (X_3 parental type)
			2.	Straw Brown.
			3.	Purple white.
			4.	Purple Brown, and
			5.	Pink white.
PLATE	14.	B.	1.	Straw - white with bluish mottlings around the eye. (X_3 parental type).
			2.	Straw Brown mottled.
			3.	Straw ash mottled.
			4.	Straw Brown, and
			5.	Pink white.
PLATE	15.	C	1.	Straw Brown mottled (X_3 parental type)
			2.	Straw - Rose white.
			3.	Straw Brown, and
			4.	Straw white.

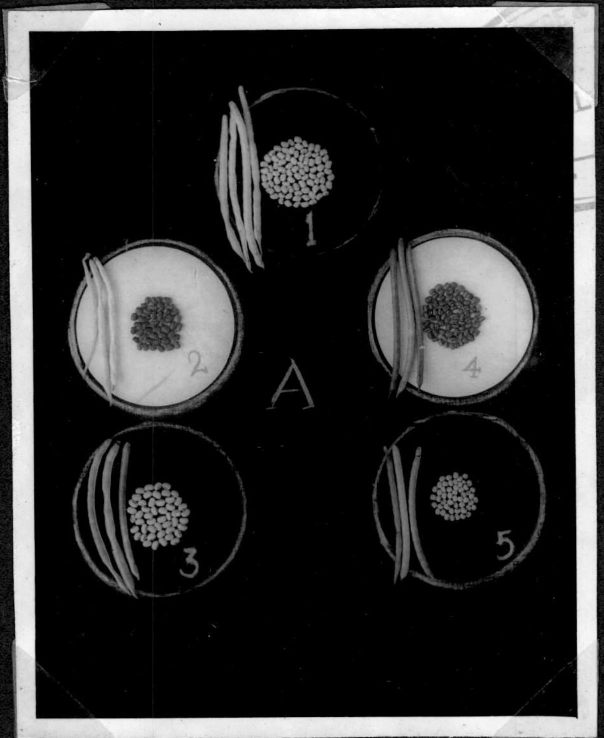


PLATE 13

PLATE 14

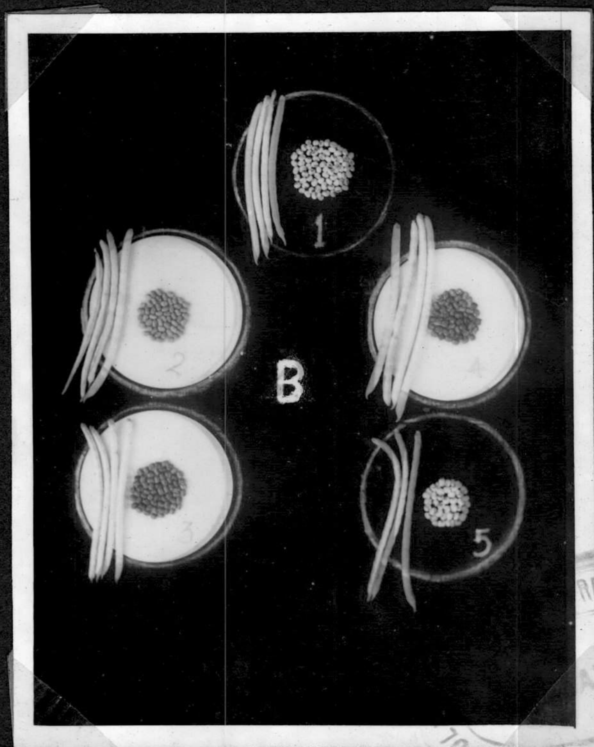
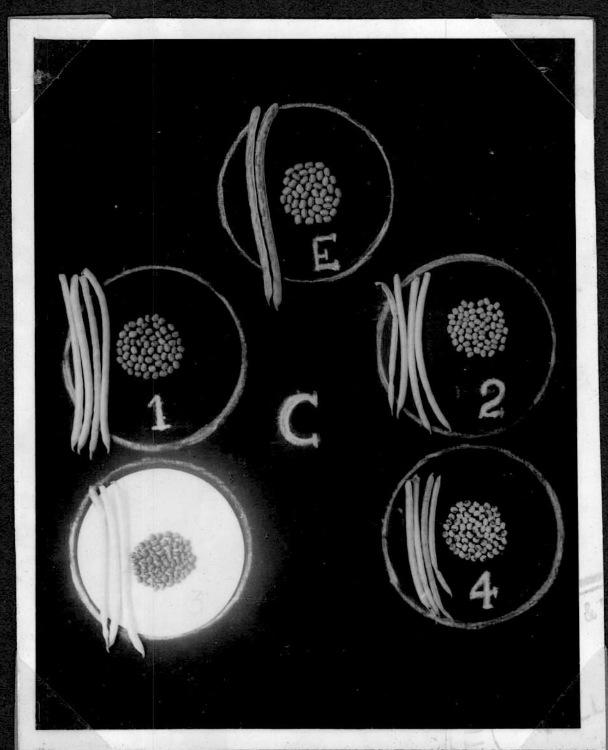


PLATE 15



Floral Abnormalities.

PLATE 16.

1. Normal - 1 standard petal 2 wing petals 1 pair keel petals (not separated).
2. 2 Standards,1 wing, 1 pair keel petals tetraddelphous, out growth from the staminal column.
3. 3 Standards, wing (nil) 1 pair keel and Triadelphous.

PLATE 17.

1. Normal flower parts .
4. 1 Standard,1 wing, 2 pairs, keel petals (not separated), triadelphous.
5. 2 Standards,2 wings, keel petals (nil), abnormal staminal column, style straight.

PLATE 18.

1 1 Standard, 2 wings, 1 pair keel petals (not separated) normal staminal column.

2 1 Standard, 2 wings, 2 pairs keel petals (not separated) normal staminal column.

3 1 Standard, 2 wings, 2 pairs keel petals (not separated) tetra adelphous.

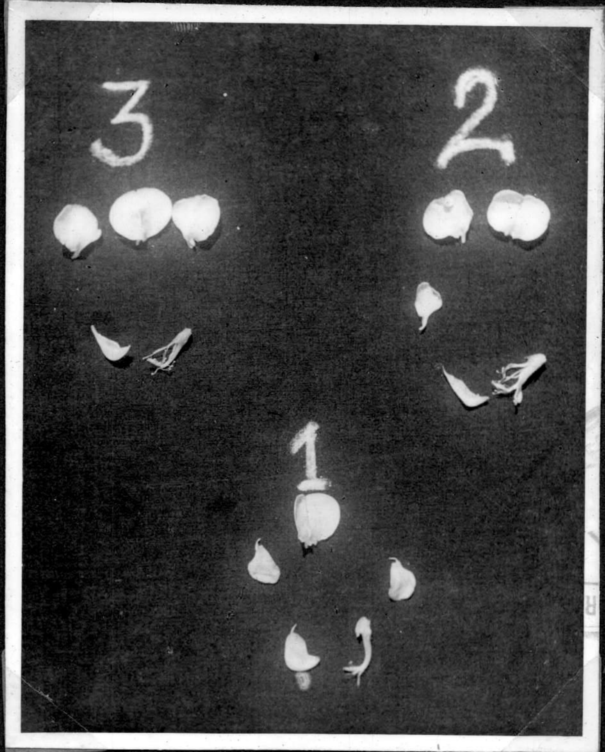


PLATE 16

PLATE 17

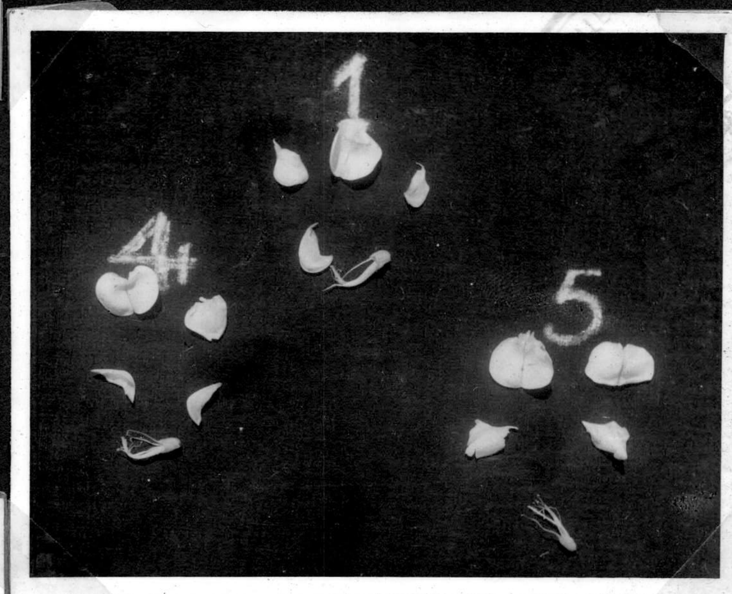


PLATE 18