STUDIES ON INDUCED MUTATIONS IN RICE (*Oryza sativa* L.)

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INTRODUCTION

INTRODUCTION

DeVries while propounding the sensational theory of mutation in his attempts to unravel the mystery of the origin and evolution of diverse biological species had conceived, as early as 1901, the idea of tailoring of better species of cultivated plants and domesticated animals by inducing mutations with X-rays and utilising them in breeding projects. Nearly quarter of a century later it was amply demonstrated that mutations could really be induced in animals and plants by exposing them to ionising radiations like X-rays. Muller (1927) while announcing his discovery of the artificial transmutation of the gene expressed the hope that this technique would be useful to plant breeders in inducing new variability. The follow up work of this fascinating, but yet amply rewarding avenue of biological research had resulted in the effective utilisation of various ionising radiations as well as radio-mimetic chemicals in the improvement of self pollinated crops through mutation breeding. The role of mutations has thus, become clearly defined as an important complementary method of crop improvement.

Studies on induced mutagenesis in plants are being conducted since the beginning of the present century for a clear understanding of the mode of action of the various physical and chemical mutagens on the biological system and of the reaction of different plant species subjected to such

mutagenic stress. Application of different kinds of mutagens has been reported to induce in varying degrees, chromosomal aberrations and gene mutations, besides effecting such biological events as germination, survival, growth and fertility in the immediate generation. In the field of mutation research, results would be proportional to the insight into the nature, induction and recovery of the mutations.

Induced mutagenesis among crop plants has been extensively studied for over fifty years in barley and wheat. These studies have enabled the choice of efficient mutagens for the purpose of inducing a very high frequency of useful and recoverable mutations. Mutation research on rice from the point of view of its improvement dates back to 1934 and has engaged the attention of a number of plant scientists particularly, in Asian countries where it forms the staple food. As pointed out by Swaminathan (1966b), the rice plant with its essentially secondary polyploid nature, with a partly diasmic and partly polysomic genetic constitution and a strict self pollinating system is ideally suited for improvement through mutation breeding. Research on induced mutations in rice breeding gained an impetus in the year 1964 when a co-ordinated programme was initiated under the direction of the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture.

Reports of early period relating to the mutation research with rice cover only such ionizing radiations as X-rays and gamma rays. The potentialities of fast neutrons however, have

not been fully explored until recently. A few studies carried out relate mostly to the assessment of its mutagenicity with reference to the induction of chlorophyll mutations. Yet, a critical comparative evaluation of both the sparsely ionising radiations and the fast neutrons at a wide range of dose levels in inducing viable morphological mutations, besides chlorophyll mutations in rice has not been made.

Information on the use of chemical mutagens in rice when compared to that of radiations is meagre. The value of highly efficient alkylating chemicals such as sulphonates and nitrosamides in mutation research has come to be recognised only recently. Their effectiveness and efficiency however, have not been thoroughly investigated.

The present investigations have been aimed at obtaining more basic information on the comparative effects of the different physical and chemical mutagens on rice and towards narrowing down the existing lacuna. For this purpose three radiations namely, gamma rays, X-rays and fast neutrons and five chemical mutagens such as diethyl sulphate, ethyl methanesulphonate, methyl methanesulphonate, nitroso methyl urea and methyl nitro nitroso guanidine were employed over a wide range of doses in a variety of indica rice.

A critical evaluation of the efficiency of the mutagens in inducing viable morphological mutations is seriously lacking in rice. This lacuna is evident in that in rice only four improved varieties have been catalogued as against a

total of 77 on the list of improved crop varieties evolved through mutation breeding (Sigurbjornsson and Micke, 1969). A comparative study of gamma rays, fast neutrons, ethyl methanesulphonate and nitroso methyl urea has been undertaken to provide information on the choice of mutagens in the improvement of rice through mutation breeding.

Studies relating to the enhancement of the rate of induction of mutations through combination and repetition of mutagenic treatments and by suitably altering the metabolic condition of the treated material by methods such as presoaking of seeds prior to treatment with chemical mutagens have not received much attention in this crop. Success in mutation breeding also depends on the knowledge of the factors influencing recovery of mutants. The extent to which a mutation induced by seed treatment may survive and give rise to a mutant is controlled by factors such as the stage of differentiation of the embryo, the number of primordial cells involved in the origin of each panicle and the magnitude of elimination of mutants through inherent or coincident lethal effects. These aspects are also given due consideration in the present investigation.

It is desirable to have information on the type and dosage of mutagens which will induce variability for economic characters governed by polygenes. The differences

in sensitivity between varieties within the subspecies *indica* of *Oryza sativa* have also not been studied in detail.

Thus, the present study with rice has been programmed to obtain additional information on the relative effectiveness and efficiency of mutagens particularly, from the point of view of induction of viable morphological mutations as well as micro-mutations and on problems pertaining to the enhancement of efficiency of induction and recovery of mutations, besides determining the differential sensitivity of its varieties to X-rays.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The idea of inducing mutations and their utilisation in breeding new forms was proposed as early as 1901 by De Vries. He stated "perhaps some day one will be able to produce permanently better species of cultivated plants and animals by mastery of mutations" (cf. Gaul, 1964a). In 1904, De Vries proposed the use of X-rays for artificial production of mutations (cf. Blakeslee, 1936). X-irradiation was applied to cells and chromosomes by Koernicke (1905) and Gager (1908). However, the conclusive proof that ionizing radiations induce mutations was presented by Muller (1927) in Drosophila. He predicted at that time that X-ray induced mutations might become important in plant breeding. Almost to the same period belong the reports of Stadler (1928a and b) on barley and maize, Gager and Blakeslee (1927) on Datura stramonium and Goodspeed (1929) on Nicotiana, that ionizing radiations can induce mutations in plants. However, after the historically important findings of this period most of the observations in the following 25 years were from investigations of a purely theoretical nature on the mutation process. In spite of many investigations, the maximum effectiveness and efficiency of radiations for inducing changes in plant characters, especially those of economic importance have not yet been realised (Wilson et al., 1965).

The search for chemical mutagens had started even before the discovery of the mutagenic effects of X-rays (Auerbach, 1967). Early in this century chemical mutagenesis was tried by Schiemann (1912). Induction of mutations by means of chemical treatments was amply demonstrated by Auerbach in England with mustard gas (Auerbach and Robson, 1942, 1947) and by Oehlkers (1943) in Germany with urethane. The investigations of Rapoport (1948) on the effects of epoxides and epimines were of a pioneering nature. Since then the number of chemicals shown to possess mutagenic properties has greatly increased and thereby the induction of mutations with chemical mutagens has made great progress in recent times.

Among the numerous radio-mimetic chemicals now known, the alkylating agents have been found to be the most efficient in a wide array of organisms from bacteria to mammals (Auerbach, 1961). Within the alkylating group, monofunctional agents in general and ethyl methanesulphonate in particular, appear to be more efficient in several organisms including higher plants (Swaminathan et al., 1962). The mutagenic efficiency of ethyl methanesulphonate was first demonstrated by Heslot et al. (1959) and Ehrenberg (1960).

A wide range of both physical and chemical mutagens is now available and it is, therefore, natural that several investigators have probed the relative advantages and disadvantages of the different mutagens (Swaminathan, 1969a and b). In sexually propagated crops chemical mutagens

have yielded very high mutation frequencies and in most cases they were more efficient than ionizing radiations (Kawre and Brunner, 1970a). However, most of the varieties developed by mutation breeding (Sigurbjornsson and Micke, 1969) have arisen from material irradiated with ionizing radiations only. It will be premature to assess the merits of chemical mutagens on the basis of the number of varieties to which they have given rise, since extensive work with chemical mutagens has begun only in 1960 following the introduction of ethyl methanesulphonate. As such, there is no definite indication that preference should be shown to either physical or chemical mutagens. Both the kinds of mutagens have their value; neutrons among radiations and ethyl methanesulphonate among chemicals are generally the mutagens of choice (Swaminathan, 1969c).

Literature on induced mutations is vast. In their bibliography, Sparrow et al. (1958) have listed the investigations relating to the period 1896 to 1955. A great deal of progress in mutation research has been achieved since this period and the results reported in a variety of journals, symposia proceedings, technical reports of panel meetings and monographs. A detailed picture has been presented in the reviews on the various aspects relating to induction of mutations by several investigators (Auerbach, 1961; Gaul, 1961b, 1964a, 1965; Sparrow, 1961; Sparrow et al. 1965;

Nilan, 1967; Nilan and Konzak, 1961; Konzak et al., 1965a; Nilan et al., 1965, 1969; Gustafsson, 1963, 1969; Gustafsson and Gadd 1965a, b, c, d, e, 1966; Heslot, 1965; Nybom and Koch, 1965; Stubbe, 1967; Narayanan and Konzak, 1969; Kawai, 1969; Scarascia-Mugnozza, 1969; Broertjes, 1969; Swaminathan, 1969a and b). The progress made in the improvement of crop plants through induced mutations is lucidly outlined by Sigurbjornsson and Micke (1969) enumerating the crop varieties released through mutation breeding. Very recently the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture have compiled and published a "Manual on mutation breeding". As such, an elaborate review of mutation research is not attempted. However, investigations on induced mutagenesis in rice are reviewed herein.

While Sparrow et al. (1958) listed only 29 publications on rice for the sixty years from 1896 to 1955, Nayar (1965) presented a review covering an additional 101 references relating to the succeeding eight years i.e., 1956 to 1963. Gustafsson and Gadd (1966) in their comprehensive review of mutations and crop improvement in rice listed about 180 references.

The earliest reports on the irradiation of rice with ionising radiations were those of Yamada (1917), Nakamura (1918) and Komuro (1919) dealing with the stimulating effects of X-rays on yield. Ichijima (1934) reported for the first time the mutations induced in rice by X-rays and ultraviolet.

Almost simultaneously in 1934, Ramiah and his coworkers initiated radiogenetical research in India (Ramiah and Parthasarathy, 1936, 1938) at Coimbatore. However, the maximum amount of work on rice radiation genetics during the early period was done in Japan.

Late in the fifties there was a revival of interest in rice mutation research. As a result, there had been a steady growth of knowledge concerning the induction of mutations with different mutagens. The investigations relating to the immediate effects of mutagenic treatments, the frequency and spectrum of mutations induced, mutagenic effectiveness and efficiency, the attempts for enhancing mutagenic efficiency, the formation of chimeras and mutated sectors, micromutations and radiosensitivity are reviewed and presented in sequence below.

I. Effects of mutagens in the M_1 generation

In mutation research, mutagenic sensitivity of plants is measured by such parameters as germination, survival, plant growth, fertility, meiotic behaviour and chlorophyll deficient chimeras.

a. Germination

Germination has been reported to be little affected by radiations though damage occured shortly afterwards (Myttenaere *et al.*, 1965; Yamagata *et al.*, 1965; Goud *et al.*, 1967; Siddiq, 1967; Ganeshan, 1970). Yamagata *et al.*, (1965) and Goud *et al.*, (1967) reported considerable delay in

germination of seeds at high doses of radiation. On the other hand germination was found to be greatly reduced by chemical mutagens such as diethyl sulphate (Rao and Ayengar, 1964), ethylene imine (Yamagata et al., 1965), ethyl methanesulphonate (Yamagata et al., 1965; Ganeshan, 1970) and nitroso methyl urea (Siddiq, 1967; Siddiq and Swaminathan, 1968a).

Sigurbjornsson and Fried (1966), Kawai and Sato (1966) and Siddiq (1967) reported that following seed irradiation there was greater reduction in growth of shoot than of primary roots. On the other hand, Kawai (1963a) with ^{32}P, Simon (1963) with neutrons and Myttenaere et al. (1965) with X-rays found that the development of root was more seriously affected than that of the shoot. Following X-irradiation, Bekendam (1961) classified seeds at germination into three groups, viz., (i) Germinated seeds with coleoptile and root developed normally, (ii) Germinated seeds with normal coleoptile but roots developed weakly or not at all, and (iii) Seeds that did not germinate.

b. Survival

The survival of seedlings was generally found to decrease with increasing doses of radiations and chemical mutagens (Rao and Ayenger, 1964; Yamagata et al., 1965; Siddiq, 1967; Siddiq and Swaminathan, 1968a; Swaminathan et al., 1970; Ganeshan, 1970). Neutrons among radiations (Siddiq, 1967; Ganeshan, 1970) and ethyl methanesulphonate among chemical

mutagens (Swaminathan et al., 1970) were found to reduce survival only slightly. Ekendam (1961) reported that following X-irradiation nearly all M_1 plants that survived up to transplanting (6 weeks) lived to the very end of the generation. The number of M_1 plants surviving to maturity was found to bear a high correlation with the number of seeds germinating with normal coleoptile and root development.

c. Plant growth

Seedling injury measured by the rate of reduction in shoot growth has been used as a reliable estimate of damage in several radiobiological experiments. Reduction in height has been more drastic generally in treatment with radiations than with chemicals (Siddiq, 1967; Singh, 1970). With gamma rays a linear relationship between dose and reduction in shoot growth was reported. Natsuo et al. (1958), Mesima and Kawai (1959) and Yamaguchi (1964) found that seedlings were less variable in height after irradiation with neutrons than with X-rays. Among chemicals nitroso methyl urea was found to be highly effective in reducing the height of seedlings (Singh, 1970). He further reported that the M_1 seedlings recovered in growth rate after 45 days. This was explained as due to the growth of uninjured meristematic cells which replaced the injured ones.

d. Fertility

A linear dependence of decreased pollen and seed

fertility on mutagen dose was reported by Beachell (1957), Chang and Hsieh (1957), Yamaguchi (1964), Siddiq (1967) and Singh (1970). Bekendam (1961), Henderson (1963) and Yeh and Henderson (1963) had indicated a decrease in fertility with increasing dose upto a certain level beyond which there was however, a saturation effect. Simon (1963), Yeh and Henderson (1963), Yamaguchi (1964) and Siddiq (1967) found that neutrons reduced fertility more severely than X-rays and gamma rays. Chemical mutagens such as diethyl sulphate (Rao and Ayenger, 1964) nitroso methyl urea and ethyl methanesulphonate (Siddiq and Swaminathan, 1968a) were reported to induce much less sterility than radiations.

The nature of M_1 sterility was interpreted to be due to chromosomal aberrations. Katayama (1963) reported that M_1 sterility induced by X-rays in rice approximately corresponded to the frequency of translocations. Singh (1970) observed that gamma rays induced a high frequency of translocations and this might be correlated with pollen sterility, whereas with chemical mutagens such as ethyl methanesulphonate and nitroso methyl urea there was a marked reduction in pollen and seed fertility though the extent of chromosomal aberrations was negligible.

e. Meiotic behaviour

Various types of induced chromosomal abnormalities were reported in rice by many workers (Chang, 1955; Carpena and Ramirez, 1960; Chao and Chai, 1961; Shastry and Ramiah, 1961; Soriano, 1961; Hsieh et al., 1962; Katayama, 1963).

Siddiq (1967), Siddiq and Swaminathan (1968a) and Singh (1970) observed translocations in radiation treatments and reported that their frequency was dose dependent. Chemicals like ethyl methanesulphonate and nitroso methyl urea did not produce extensive breakages. Bridges and laggards were observed following gamma irradiation but they were rare in chemical treatments.

f. Chimeras

Chlorophyll deficient sectors on the M_1 plants of rice following irradiation were observed by Shastry and Hsieh (1961), Horvat (1961) and Siddiq (1967). Tanaka (1970) recorded such sectors in haploid plants following chronic gamma irradiation. But the frequency of plants with sectors did not show a clear dependence on dose. Plants with chlorophyll deformities were observed following treatment with chemical mutagens, such as ethyl methanesulphonate and nitroso methyl urea by Siddiq (1967) and Singh (1970). Hsieh (1959) suggested that the variegations induced by radiations in the M_1 generation were due to plastid mutations. Siddiq (1967) found them to be nonheritable and indicated the possibility of their being either periclinal chimeras or arising from a physiological disorder.

Tanaka (1970) also observed diploid-like sectors on haploid plants subjected to chronic gamma irradiation. The frequency of haploid plants with diploid-like sectors increased with increasing dose.

II. Mutations in the M_2 generation

a. Chlorophyll mutations

i) Frequency

An increase in the frequency of chlorophyll mutations with increasing doses of mutagens was reported by several investigators in rice. Kawai and Sato (1966) following X-irradiation found an increase in mutation frequency with an increase in M_1 injury. With X-rays and gamma rays the mutation frequency reached a maximum at moderate doses and decreased at high doses (Matsuo et al., 1958; Masima and Kawai, 1959; Osone, 1960; Yamaguchi, 1964; Miah et al., 1970). On the other hand, an exponential increase in frequency with an increase in dose was reported by Ekendam (1961), Siddiq (1967) and Siddiq and Swaminathan (1968a). A linear relationship between mutation frequency and dose of X-rays and gamma rays was reported by Yamaguchi and Miah (1964) and Singh (1970). Following neutron irradiation the frequency of mutations was found to increase with increasing dose (Matsuo et al., 1958; Masima and Kawai, 1959; Yamaguchi, 1964).

There was however, no significant difference in yield of mutations between X-rays and gamma rays (Yamaguchi and Miah, 1964). On the other hand, neutrons were reported to yield a higher frequency of chlorophyll mutations than sparsely ionizing radiations by Matsuo et al. (1958); Masima and Kawai (1959), Matsuo and Onozawa (1961), Kawai (1968a).

Mikaelsen et al. (1968) and Miah et al. (1970). Soriano (1968) obtained much higher mutation frequencies with fast neutrons than that reported previously in rice with thermal neutrons. Swaminathan et al. (1970) also reported high mutation frequencies following irradiation with fast neutrons.

Several chemical mutagens were found to be highly effective in rice. High chlorophyll mutation frequencies were reported with ethylene imine (Yamagata et al., 1965; Kawai and Sato, 1965), diethyl sulphate (Gopal-Ayengar et al., 1969), ethyl methanesulphonate (Swaminathan, 1966a; Ismail, 1969), nitroso methyl urea (Swaminathan, 1966a; Siddiq, 1967) and methyl nitro nitroso guanidine (Swaminathan et al., 1968, 1970).

ii) Spectrum

Differences in the spectrum of mutations induced by physical and chemical mutagens were reported by several investigators. Albina was generally the predominant type of mutant met with. This might be due to very large number of loci governing this phenotype (Swaminathan et al., 1970). Among the radiations, fast neutrons induced the widest spectrum of chlorophyll mutations (Swaminathan et al., 1970). Ethyl methanesulphonate was found to induce a wider spectrum of mutations than the radiations (Swaminathan 1966b). Bekendam (1961), Chao and Chai (1961), Matsumura and Mabuchi (1964), Kawai (1966) and Basu and Basu (1969) reported that following irradiation, the albinas predominated the chlorophyll

mutation spectrum followed by viridis and xantha, whereas in treatment with chemical mutagens such as ethyl methanesulphonate and diethyl sulphate, xantha and viridis mutants were found to increase with a proportionate decrease in albinas (Sato, 1966). However, the spectrum of chlorophyll mutations was reported to be independent of the mutagen used by Kawai and Sato (1965), Siddiq (1967) and Siddiq and Swaminathan (1968a). Kawai and Sato (1965) did not find any difference between the spectra of different doses of the same mutagen.

iii) Multiple mutations

Siddiq (1967) reported that over 94 per cent of the ear progenies segregated in the M_2 generation only for one type of mutation following irradiations with gamma rays and neutrons. On the other hand, less than 90 per cent segregated for one type following treatment with chemical mutagens such as ethyl methanesulphonate and nitroso methyl urea, thereby indicating the comparatively high efficiency of the chemical mutagens to induce more than one mutational event in one and the same ear primordium.

b. Viable mutations

i) Frequency and Spectrum

A high frequency of viable mutations in the M_2 generation was reported by Singh (1970). Siddiq (1967) observed that the frequencies clearly indicated a saturation effect. According to Kawai (1968a) the frequency was higher

after irradiation with pile neutrons than with X-rays. Siddiq (1967) was of opinion that at comparable doses of ethyl methanesulphonate and gamma rays the frequencies of viable mutations were more or less the same; whereas Singh (1970) reported that ethyl methanesulphonate was the most potent mutagen.

A wider spectrum of viable mutations was reported after treatment with chemical mutagens such as ethyl methanesulphonate (Siddiq, 1967; Siddiq and Swaminathan, 1968a) and nitroso methyl urea (Singh, 1970) than that obtained with radiations.

Large collection of viable mutants was reported by several workers. More than 1000 types were induced in a single variety by radiation treatments as reported by Kawai (1968b). About 1400 mutant lines with stable visible characters isolated from gamma irradiation of growing plants were assembled by Tanaka (1969). Nekendam (1961) also reported a large number of mutant types. Relatively smaller groups include 478 types induced with ^{32}P by Kawai (1963a), 287 types with X-rays by Kawai (1963b), 283 types with ^{32}P by Masima and Kawai (1958), 254 types by Ouang (1964), 121 types by Marie (1967), 66 types by Swaminathan et al. (1970) and 35 types by Viado et al. (1970)

Many mutants of economic value have been reported to be induced in rice by Bora and Rao (1958), Hu et al. (1960),

Bekendam (1961), Li et al. (1962) and Matsuo and Yamaguchi (1962). According to Marie (1970) useful types form a minority among the viable mutants isolated. Gustafsson and Gadd (1966) also concluded that approximately one or two out of 100 mutants isolated might be of value in plant breeding. However, Tanaka (1969) stated that the use of mutations for rice improvement was extremely effective. A number of promising mutant types were evolved in Japan and Taiwan and four mutant varieties were already released for commercial cultivation (Sigurbjornsson and Micke, 1969).

ii) Types of mutations

In their monograph, Ramiah and Rao (1953) summarised all cases of spontaneous and induced mutants in rice isolated upto that time. Mutations affecting one or several morphological characters have been realised in various studies with indica, japonica and javanica varieties of rice. They may be broadly grouped into three types based on the scheme of classification proposed by Swaminathan (1964) such as (1) Macro-mutations, (2) Visible mutations, and (3) Systematic mutations.

1) Macro-mutations

Mutations affecting more than one character of the same plant were reported in many investigations. These simultaneous changes were inherited as a single unit of recombination. The most important of these macro-mutations

were the erectoides mutants and these were characterised by short stature, stiff straw, broad and dark-green leaves, compact ear and small grains. They could be induced with radiations in both indica and japonica varieties (Li et al., 1962, 1966a). Erectoides mutants were also reported by Hasima and Kawai (1958), Hu et al. (1960), Matsuo and Onozawa (1961), Li et al. (1961) and Kawai (1963b). They were found to differ from one another in many minor characters and some of them were higher yielding than the original varieties (Li et al., 1968).

The simultaneous effects on a number of characters in macro-mutants were attributed to the pleiotropic action of a single mutated gene (Kawai, 1963a, 1968b; Nanahari, 1970b; Joshua et al., 1970). However, Nee (1970) explained this to be due to the compound effect of two or more neighbouring, absolutely linked genes which changed simultaneously during the mutagen treatment.

2) Visible mutations

Mutations affecting various plant characters have been induced with a variety of radiations and chemical mutagens.

Culm length: Mutants with reduced height were reported to be of more common occurrence than those with increased height by Kawai (1962) and Tanaka (1968). Genetic dwarfness varied in appearance from the most extreme stuntedness to semi-dwarfs (Gustafsson and Gadd, 1966). Semi-dwarfs

(short culm) and dwarfs have been reported by numerous investigators such as Misima and Kawai (1958), Kawai et al. (1961), Bekendam (1961), Matsuo and Onozawa (1961), Hsieh (1962), Narahari and Bora (1963), Kawai (1963b), Shastry (1965), Shastry and Madhachary (1965), Swaminathan (1966a), Siddiq (1967), Siddiq and Swaminathan (1968a), Ismail (1969), Singh (1970), Mish et al. (1970) and Ganeshan (1970).

Maturity: Yamagata (1964) reported that in rice the mutation rate for maturity was very high. He concluded that numerous loci might be responsible for heading time. On the other hand, Tanaka (1968) observed that mutants for duration showed the least frequency among the viable mutations. Lateness was reported to be more frequently induced than earliness (Kawai, 1962, 1963a; Sato, 1966). Yamagata (1964) and Matsuo and Yamaguchi (1967) concluded that a higher frequency of early mutants would be induced in a late variety and vice versa. Singh (1970) found early types to be more common in the late variety but the converse was not true. Bekendam (1961) recorded a late flowering mutant in which flowering was delayed by a period of three months. He inferred that the late flowering might not be solely induced by photoperiodism. Mutants with altered duration were isolated by Bora and Rao (1958), Jodon (1958), Bekendam (1961), Li et al. (1962), Kawai (1963a), Simon (1963), Ouang (1964), Bilquez et al. (1965), Cada et al. (1966) and Mish and Bhatti (1968).

Leaf types: Mutations affecting leaf characters such as size, shape, arrangement, texture, pubescence and colour were isolated. Narrow leaf mutants were the most frequent as reported by Hsieh (1962), Shastry (1965), Siddiq (1967), Tanaka (1968), Singh (1970) and Swaminathan et al. (1970). Boat leaf mutants have been reported under different names like incurved lamina or rolled leaf by Narahari and Bora (1963), Tanaka (1968), Singh (1970) and Swaminathan et al. (1970).

Ear types: Tanaka (1968) found short panicle mutants to be more frequent than long panicle mutants, and mutants with lax panicles to be as frequent as those with compact panicles. Singh (1970) also reported that mutations affecting panicle density, such as compact and open types, were common.

Grain types: Mutations observed in the nature of the grains were in relation to size, shape, colour, awning, pubescence and beaking. Nagina and Kawai (1958), Kawai (1962) and Ganeshan (1970) reported that short grain types were more frequent than long grain types. Mutants with altered grain size and shape were recorded by Beachell (1957), Bora and Rao (1958) and Syakudo et al. (1959). Hsieh (1962) obtained a tawny glume mutant and Siddiq (1967) recorded several mutants with dark brown glumes. Awned mutants were reported by Soriano (1961) and Narahari and Bora (1963). Tip awned mutants were found to be more frequent than fully awned ones (Siddiq, 1967).

Defective types: The most distinct change of growth habit observed in mutants is ageotropism or laziness (Kawai, 1970). Lazy mutants were reported by Ramiah and Parthasarathy (1936), Jones and Adair (1938) and Hsieh (1962). A mutant with neck leaf was recorded by Jones (1952). Clustered spikelets were observed by Coyaud (1950). A number of mutations for spikelet abnormalities were induced with X-rays by Ramiah and Parthasarathy (1938), with neutrons by Nanahari and Bora (1963) and with gamma rays, neutrons and ethyl methanesulphonate by Ganeshan (1970). Mutants having spikelets with long sterile glumes (winged) have been recorded by Kadam et al. (1941), Nanahari and Bora (1963), Ganeshan (1970) and Swaminathan et al. (1970). Oka (1963) found a mutant with inner glumes shorter than the outer ones. This character did not appear in all the spikelets of a panicle. Open spikelet mutants were reported by Nanahari and Bora (1963) and Siddiq (1967). Gill et al. (1969) recorded an open spikelet mutant with two types of spikelets in the same panicle i.e., open and sterile spikelets and open spikelets with partially developed kernels. Claw hull and triangular hull types have also been recorded (Chandraratna, 1964).

Viable chlorophyll mutants: Some of the chlorophyll mutants such as striata, chlorina, tigrina and virescent were found to be viable with normal fertility by Tanaka (1968) and Vinde (1968).

3) Systematic mutations

These mutations either simulate an existing taxon or necessitate the creation of a new systematic unit. Shastry (1965) isolated a dwarf in Oryza sativa resembling the wild species O.granulata. Swaminathan (1966b) also obtained a similar type and also a mutant in O.glaberrima resembling O.rufipogon. Siddiq and Swaminathan (1968a and b) and Swaminathan et al. (1968) isolated stable mutants affecting the key characters that usually distinguish japonica and indica varieties. In each of these two groups several mutants with the phenotypic characteristics of the other group were induced. Such mutants occured only in treatment with ethyl methanesulphonate and not with radiations.

c. Chlorophyll Vs. viable mutations

The frequency of viable mutations was found to be parallel to that of chlorophyll mutations following treatment with radiations and chemical mutagens by Matsuo and Onosawa (1961) and Sato (1966). Progenies segregating for chlorophyll mutations in the M_2 generation were found to yield viable mutations more often than progenies not segregating for chlorophyll mutations. Siddiq (1967) however, did not find any such relationship and concluded that their manifestation was independent of each other.

d. Mutagenic effectiveness and efficiency

Siddiq (1967), Siddiq and Swaminathan (1968a) and Swaminathan et al. (1970) reported that among radiations

neutrons are the most effective. Soriano (1968) found that fast neutrons induced higher mutation frequencies than thermal neutrons. Rao and Ayenger (1964) reported higher mutation frequency with diethyl sulphate than with thermal neutrons and gamma rays. Japanese investigators have repeatedly confirmed the high potency of ethyl methanesulphonate and ethylene imine in comparison with sparsely ionising radiations such as X-rays (Kawai and Sato, 1965; Yamagata et al., 1965; Sato, 1966; Matsuo and Yamaguchi, 1967). On the other hand, Swaminathan (1966a) and Siddiq and Swaminathan (1968a) found gamma rays to be as effective as ethyl methanesulphonate. High mutagenic effectiveness of nitroso methyl urea was reported by Swaminathan (1966a), Siddiq (1967), Siddiq and Swaminathan (1968a) and Singh (1970) and that of methyl nitro nitroso guanidine by Swaminathan et al. (1968, 1970) and Siddiq and Swaminathan (1968c). With respect to mutagenic efficiency, ethyl methanesulphonate was found to be the best mutagen probably because of its less injurious effects on survival and fertility in the M_1 generation. Swaminathan et al. (1970) concluded that chemicals have no particular advantage over ionising radiations with reference to either mutation frequency or spectrum.

Siddiq (1967), Siddiq and Swaminathan (1968a and c), Swaminathan et al. (1970) and Singh (1970) have reported that mutagenic effectiveness as well as efficiency decrease with increasing doses of mutagens. This was explained to be due

to a higher rate at which damage increases than mutations with increasing doses.

e. Relative biological effectiveness (RBE)

According to Fujii (1962) the RBE of X-rays and that of gamma rays are approximately the same. A number of investigators have found that neutrons are several times more effective than sparsely ionizing radiations. Siddiq (1967) has reported that irrespective of the criteria used for comparison, neutrons have higher RBE values. According to Kawai (1968a) the values differ for the different criteria such as germination, seedling growth, survival, fertility and mutation frequency. Mikaelsen et al. (1968) and Mikaelsen and Navaratna (1968) found the RBE of fast neutrons in relation to gamma rays for growth reduction and survival to be 10 and for M_1 fertility to be 17. Matsumura (1964) calculated the RBE values to be 30 to 50 for M_1 seed fertility and M_2 chlorophyll mutation frequency. Siddiq (1967) observed that the RBE values were the lowest for seed fertility and highest for germination.

f. M_1 fertility and M_2 mutations

Nekendam (1961) found that the frequency of chlorophyll mutations was low in both the lowest and highest fertility classes. The decrease in the lowest class was explained to be due to elimination of mutations, and that at the highest class to be due to diplontic selection. The distribution of

albina and viridis mutants seems to be the same over the different fertility classes. Kawai (1963b) obtained a large number of morphological mutants in progenies of M_1 plants with low fertility. Ando (1968) reported a decrease in the segregation ratio with an increase in fertility.

g. Genetics of mutants

According to Kawai (1963b) mutants are particularly suitable for the study of gene interactions because the action of genes studied is not influenced by other genes. Most of the induced mutants in rice are recessive to the normal types. Li et al. (1965, 1966a, b, 1968) and Hu et al. (1970) report that the induced erectoides mutants are controlled by the same gene and this locus might have a multiple allelic series. The induced dwarf mutants (Hsieh, 1962) and early mutants (Yamagata, 1964) have been reported to be recessive. Claw hull (Takahashi, 1950), triangular hull (Morinaga and Fukushima, 1943), open spikelet (Gill et al., 1969) and winged spikelet (Butany and Bhattacharya, 1962) are also reported to be monogenic recessives.

A dominant mutant for blast resistance was reported by Tanaka (1969) and a few completely or partially dominant mutants were reported by Kawai (1963b).

III. Attempts for enhancing mutagenic efficiency

a. Combination of mutagens

Matsuo and Onozawa (1961) found that diepoxy butane in combination with X-rays was effective in increasing fertility but was not effective in combination with thermal neutrons. Rao and Ayengar (1964) observed that the toxicity of diethyl sulphate was reduced in post treatment with thermal neutrons but not with gamma rays. According to Soriano (1968) ethyl methanesulphonate applied after fast neutron irradiation did not affect seedling height and fertility, whereas methyl methanesulphonate in combination with fast neutrons led to increased effects.

A synergistic effect on mutation frequency in combination treatments of diethyl sulphate with thermal neutrons as well as gamma rays was reported by Rao and Ayengar (1964). Gamma rays and ethyl methanesulphonate in combination produced more chlorophyll mutations than individual treatments, but the frequency was less than additive (Swaminathan, 1966a). Soriano (1968) found that the mutation frequency in the combination of fast neutrons and ethyl methanesulphonate was additive. The combination of fast neutrons and methyl methanesulphonate was on the other hand inefficient.

In combinations of radiations and diethyl sulphate there was a decrease of albina mutants and an increase of viridis as compared to radiations alone (Rao and Ayengar, 1964).

b. Recurrent treatments

Swaminathan (1966a) has stated that *indica* varieties have more than one gene governing several traits and in such cases recurrent irradiation has an unmasking effect on the external manifestation of the characters controlled by duplicate factors. However, Siddiq and Swaminathan (1968d) have found a reduction in mutation frequency in the recurrently irradiated population.

c. Presoaking of seeds before mutagenic treatments

Increase in radiosensitivity of seeds following presoaking in water was reported by many investigators (Syakudo and Yamagata, 1957; Ota *et al*., 1957; Matsuo *et al*., 1958; Yamagata and Syakudo, 1960; Shastry and Ramiah, 1961; Kawai and Sato, 1966). Matsuo *et al*. (1958) found that presoaking of seeds did not increase sensitivity to neutrons, as it did with X-rays. Yamaguchi (1969b) observed that following gamma irradiation, survival and fertility gradually decreased up to 58 hours of soaking and then increased. On the other hand, Shama Rao (1970) found that seedling height progressively decreased with X-irradiation of seeds presoaked from zero to 96 hours.

Presoaking was reported to increase sensitivity to chemical mutagens such as diethyl sulphate, ethyl methanesulphonate and nitroso methyl urea by Ando (1968), Ismail (1969), Gopal-Ayengar *et al*. (1969), Swaminathan *et al*. (1970), Siddiq *et al*. (1970) and Shama Rao (1970). A reduction

in seedling height following presoaking upto 56 hours prior to treatment with diethyl sulphate and ethyl methanesulphonate was observed, beyond which there was a gradual recovery upto 72 hours (Shama Rao, 1970). The data presented by Gopal-Ayengar et al. (1969) indicated that survival declined with increasing periods of presoaking upto 40 hours. In dehulled seeds, Swaminathan et al. (1970) found a drastic increase in sensitivity after presoaking for 18 to 22 hours. Ismail (1969) reported that the points of sensitivity peaks remained more or less at identical hours of presoaking in the case of germination, survival and seedling injury, but were different in the case of fertility.

Kawai (1962) found the mutation frequencies to be high when seeds were soaked prior to irradiation with X-rays or gamma rays. Yamaguchi (1969a and b) observed a gradual increase in mutation frequency with increasing soaking time with a maximum frequency at 58 to 60 hours of soaking and a decrease thereafter. On the other hand, Gopal-Ayengar et al. (1969) reported that the mutation rate was more or less constant upto 24 hours of presoaking with a sharp rise during the 24 to 32 hour period, and a further increase beyond 32 hours reaching a maximum at 48 hours. In seeds without hull there was a sudden increase in mutation frequency at 12 to 16 hours of presoaking and a further increase thereafter reaching a maximum at 24 hours. Ismail (1969) found in seeds presoaked beyond 20 hours a higher mutation frequency with prominent peaks at

21 to 24 hours. Siddiq et al. (1970), however, recorded four major peaks at 11, 18, 22 and 26 hours of presoaking.

Yamaguchi (1969a) reported that the segregation ratio in M_1 ear branch progenies was reduced to half, in the period of 48 to 72 hours after presoaking, indicating that the initial cell of the ear branch primordia had already divided into two. Studies with tritiated thymidine reveal that synthesis of DNA, in the cell from which germinal tissue originates, occurs approximately 60 hours after the beginning of seed soaking. The clear peak in sensitivity as judged from lethality, sterility and mutation frequency at 58 hours after presoaking was explained by Yamaguchi (1969b) to be due to the first round of DNA replication in the embryo cells at that time. Autoradiographic studies by Gopal-Ayengar et al. (1969) have revealed that DNA synthesis is initiated between 24 and 32 hours in seeds of rice with hull and between 12 and 16 hours in seeds without hull and continues beyond 48 hours and 24 hours in the two cases respectively. Thus, in both categories of seeds the sensitivity as judged from M_1 survival and M_2 mutation frequency synchronises with DNA synthesis. In seeds without hull, Ismail (1969) and Siddiq et al. (1970) also have found that the sensitive phase at 18 hours coincides with the beginning of S phase of DNA synthesis.

Differences in the DNA synthesis periods in the reports of Gopal-Ayengar et al. (1969) and Yamaguchi (1969b) might be due to subspecies differences and due to differences in

environmental factors during treatment time affecting the rate of metabolic activity in seeds as indicated by Shama Rao (1970). The reports of Mikaelsen et al. (1968) that the onset of DNA synthesis in germinating seeds depended very much on temperature and of Goud et al. (1967) that indica types germinated faster than japonica, lend support to this statement.

The results reported by Ismail (1969) revealed that presoaking of seeds for varying periods brought about a distinct alteration in the spectrum of chlorophyll mutations. Siddiq et al. (1970) also reported that ethyl methanesulphonate induced albina and nitroso methyl urea induced viridis mutants with higher frequencies at short periods of presoaking, whereas both the chemicals induced xantha mutations at longer periods of presoaking. These results were in support of the view of Swaminathan and Sharma (1968) that by treating with mutagens during different stages in the S phase of DNA synthesis, the relative proportion of different types of mutations could be altered.

d. Effect of hull

Several chemical mutagens have proved to be effective in barley and wheat but not in rice, presumably because of the presence of the hull or the inability of the chemical to penetrate the water resistant corky layer of the bran (Mikaelsen and Naveratna, 1968). Siddiq et al. (1968) and Gopal-Ayengar et al. (1969) reported that the diffusion of the chemical mutagen was slow in seeds with hull and that the hull

hindered their easy and rapid uptake. Siddiq and Swaminathan (1968a) and Konra and Brunner (1970b) found that dehulling accelerated the rate of mutagen diffusion. Swaminathan (1966a), Gopal-Ayengar et al. (1969), Swaminathan et al. (1970) and Singh (1970) had reported that dehulling of seeds increased the efficiency of the chemical mutagens by enhancing the frequency of mutations.

It was observed that DNA synthesis took place in seeds without hull nearly 12 to 16 hours earlier than in seeds with hull. The mitotic indices also revealed that the initiation of division in seeds without hull (16 hours onwards) was earlier than in seeds with hull (32 hours onwards) by 16 hours (Gopal-Ayengar et al., 1969).

IV. Chimera formation and mutated sectors

Radiobiological studies present a new line of approach in investigations on the structure of the embryo and development of plant organs through the differential effects on the primordial cells.

a. Chimera formation

Imai and Kaschara (1937) noticed the chimeric nature of M_1 plants and reported that all panicles of an M_1 plant were not heterozygous for a mutation. The independence of the occurrence of mutations in M_1 ears was studied by Nishimura and Kurakami (1952), Matsuo et al. (1958), Toriyama and Futsuhara (1959), Kawai (1962, 1963a), Osone (1963).

Siddiq (1967) and Siddiq and Swaminathan (1968a). The results of these investigations demonstrated that the ears of primary tillers as far as the fifth from the lowest might mutate independently of the ear of the main stem. Ears of primary tillers from upper node and the ear of the main stem often showed the same kind of mutation. Secondary tillers were usually found to give the same kind of mutations as the primary tillers from which they arose. This was in conformity with the concept of mutational clusters proposed for barley by Jacobsen (1966). Swaminathan (1966a) inferred that the dormant rice seed when exposed to mutagenic treatment had 8 to 10 initials which would give rise to tillers. Kawal (1963a) reported that following treatment with heavy doses of radiations, the same mutation was distributed over almost all the ears in certain M_1 plants, indicating that all the ears originated from a single primordium. It was inferred that all primordia except one might have been killed by severe radiation injury.

Osone (1961) and Bekendam (1961) found that the mutation rate was highest in the ear progeny of the main stem and decreased with later emergence of tiller. Osone (1963) further clarified that on an average the mutation rate decreased with the order of primary tillers and the order of secondary tillers. The secondary tillers gave lower rates than the corresponding primary ones.

b. Segregation ratios

Nishimura and Kurakami (1952) and Yamaguchi (1959)

reported that the frequencies of mutants in mutated M_1 ear progenies (segregation ratios) were smaller than 25 per cent, the expected frequency for single recessive mutations. Osone (1961, 1963) and Kawai (1963a) had observed that the segregation ratio also changed with the order of tillers. The ratio was smallest in the ear progeny of the main stem and increased in the following order i.e., primary tillers from lower nodes, primary tillers from upper nodes and secondary tillers.

According to Yamaguchi (1964) the segregation ratios were larger with X-ray irradiation as compared with neutrons. Kawai and Sato (1965) reported that the segregation ratio was small in treatment with ethylene oxide and ethylene imine than with X-rays. Siddiq (1968) and Siddiq and Swaminathan (1968c) found lower segregation ratios with ethyl methanesulphonate than with gamma rays. But Siddiq and Swaminathan (1968c) found higher segregation ratios following treatment with methyl nitro nitroso guanidine. However, Swaminathan et al. (1970) had concluded that chemical mutagens generally yielded a lower segregation ratio than radiations, probably due to the operation of a less rigorous somatic sieve.

Yamaguchi (1962a) demonstrated the dependence of M_2 segregation ratios on the dose of a mutagen. Following gamma irradiation the segregation ratios increased with increasing doses. Kawai and Sato (1965) and Ando (1968) also reported an increase in segregation ratios with increasing dose of mutagens.

c. Size of mutated sectors

When the M_1 ears are genetically uniform, the M_2 segregation ratios are normally expected to be equal to the inherent segregation ratios of induced mutations. The low ratios indicate that the induced mutation occurs only in a sector of the M_1 ear and the size of the mutated sector may be proportional to the segregation ratio. The greater number of seeds per ear make rice a favourable material for the study of mutated sectors (Yamaguchi and Miah, 1964).

Bekendam (1961) and Osone (1963) estimated the size of the mutated sector of the M_1 ear by dividing the M_2 segregation ratio with 20 per cent, the expected average segregation ratio of recessive mutants as reported by Moh and Smith (1951). Siddiq (1968) on the other hand adopted the value of 25 per cent for the expected ratio in calculating the size of the mutated sectors. Kawai and Sato (1965) made the best estimate of mutated sector size by dividing the M_2 segregation ratio with the corresponding M_3 ratio. The estimations were confined to progenies where the number of recessive mutants was nearly one half of heterozygotes in the M_2 generation.

Kawai and Sato (1965) and Siddiq (1968) observed that the size of mutated sectors in M_1 ears was smaller in treatment with chemical mutagens than with radiations. A dependence of mutated sector size on the dose of the mutagen was reported by Bekendam (1961), Yamaguchi (1962a), Osone (1963), Kawai and Sato (1965), Siddiq (1968) and Singh (1970). The sector

size was found to increase with increasing doses of chemical mutagens and radiations. This increased sector size at higher doses was probably due to a proportionate reduction in the number of initial cells involved in the development of the panicle. The initial cells in a group would be disturbed independently by chromosome aberrations as well as point mutations. If the mutated cell also contained a chromosomal aberration leading to death, it got eliminated. If on the other hand, some of the nonmutated cells contained the aberration the size of the mutated sector increased (Osone, 1963).

d. Number of initial cells

The multicellular nature of germinal layer has been reported by several investigators in rice (Shastry and Ramiah, 1961; Bekendam, 1961; Yamaguchi, 1962a; Osone 1963; Kawai, 1963a; Kawai and Sato, 1965; Siddiq, 1968). The number of initial cells contributing to the germinal tissue of a panicle is inversely proportional to the size of the mutated sector and can be estimated from the M_2 segregation ratios (Kawai 1963b; Kawai and Sato, 1965). Bekendam (1961) and Siddiq (1967) have estimated that the generative tissue of a single panicle may derive from one to four cells. According to Osone (1963) the number of cells in the embryo initials forming the main panicle is five to six on an average.

Osone (1963) concluded that the generative tissue of the later formed tillers was derived from a smaller number of initial cells than that of the main stem. The later

formed primary tillers and the secondary ones were not always represented by definite initial cells. Yamaguchi (1963) and Yamaguchi and Miah (1964) assumed that the primary branch of an M_1 ear could be traced back to a single cell in the rice embryo. The number of initial cells affected the mutation frequency and segregation ratio of the ear. The smaller the number of initial cells the lesser the probability for a mutation to occur in them and the larger the size of the mutated sector, implying that the later formed ears would have low mutation frequency but high segregation ratios. This inference was confirmed by the observations of Frydenberg et al. (1964) in barley.

e. Deficit and excess of mutants

The observed M_3 segregation ratios were sometimes significantly lower than 25 per cent, suggesting an inherent recessive deficit for chlorophyll mutations in rice. Recessive deficit seems to be more frequent than recessive excess (Kawai and Sato, 1965). Rekendam (1961) inferred that the deficit of recessive mutants in advanced generations in rice was of the same magnitude as reported by Moh and Smith (1951) for barley and wheat. Deficit of recessive chlorophyll mutants was also reported by Yamaguchi (1962a), Kawai and Inoshita (1965) and Siddiq (1968). The low frequency of recessives was explained by Kawai and Sato (1965) and Kawai and Inoshita (1965) to be due to (1) elimination of either or both male and female gametes carrying mutations (2) low viability of homozygous mutant zygotes or proembryos, and (3) low germinability of seeds with homozygous

mutant genes. By critical estimations, Kawai and Sato (1965) found that in segregating progenies the recessive homozygous mutants were less than one half of the heterozygous ones, indicating zygote or proembryo elimination.

Kawai and Sato (1965) and Kawai and Inoshita (1965) reported in the M_3 generation segregation ratios with more than 25 per cent of recessives; the ratios in certain cases were as high as 59 and 56.5 per cent respectively. Simultaneous induction of two or more mutations of the same phenotype has been suggested as the possible reason for high segregation ratios by Kawai and Inoshita (1965).

V. Micro-mutations

The expression "micro-mutation" is used to mean mutations in polygenes governing quantitative traits leading to small changes in phenotype. The first analysis of induced mutations for quantitative traits in rice was published by Oka et al. (1958). Several investigators have reported that the mean values in respect of various quantitative characters were not altered significantly following treatment with radiations as well as chemical mutagens (Oka et al. 1958; Matsuo and Onozawa, 1961; Ota et al., 1962; Kawai, 1962; Yamaguchi, 1964; Miah and Bhatti, 1968; Sharma and Saini, 1970). On the contrary, Matsuo et al. (1964) found significant differences in mean values for length of panicle between treated and control populations. Bateman (1959) also pointed out that there was increase in means following mutagenic treatments. Sakai and

Suzuki (1964) reported that the means of treated populations in the M_4 generation depended on the character. Miah and Yamaguchi (1965) found that with respect to grain size the irradiated population had the same mean as the control, whereas for endosperm quality the mean was higher. Singh (1970) observed that the mean values either remained the same or were shifted. The means were lower with respect to height and flowering duration but higher for number of tillers and plant yield.

Increase in variance following mutagenic treatments was a common feature observed in quantitative characters as reported by several investigators (Oka et al., 1958; Bateman, 1959; Kao et al., 1960; Matsuo and Onozawa, 1961; Kawai, 1962; Chari, 1963; Matsuo et al., 1964; Sakai and Suzuki, 1964; Yamagata, 1964; Yamaguchi, 1964; Miah and Yamaguchi, 1965; Miah and Bhatti, 1968; Sharma and Saini, 1970; Singh, 1970). Oka et al. (1958) stated that for a constant dose of radiations the genetic variance induced was more for height of plants than for heading date.

Matsuo and Onozawa (1961) reported that the increase in variance was more in the case of radiations than with diepoxy butane. Moreover, neutrons were found to induce larger variance than X-rays. Oka et al. (1958) and Ota et al. (1962) reported an increase in variance with increasing doses of the mutagens but Yamaguchi (1960) observed a saturation

effect. Yamaguchi (1964) confirmed that variance did not increase linearly with the radiation dose. On the other hand, Kao et al. (1960) and Mish and Bhatti (1968) reported that the variance decreased at higher doses.

The symmetrical increase of variability without significant difference in the mean values made Oka et al. (1958), Matsuo and Onozawa (1961), Yamaguchi (1964) and Sharma and Saini (1970) suggest that mutations with positive and negative effects occured with approximately equal frequency. On the other hand, Sakai and Suzuki (1964) and Tanaka (1968) found that the distribution of variance for certain characters was skewed and therefore, stated that the mutation of polygenes occoured mostly in a negative direction. Swaminathan (1966b) was of opinion that the directions of incidence of micro-mutation was strongly influenced by the previous selection history of the variety.

VI. Radiosensitivity

Fujii (1962) recorded that the allopolyploid species Oryza latifolia, O.alta and O.minuta, and the diploid species O.glaberrima and O.sativa f.spontanea were highly radioresistant. Radiosensitivity in rice, varies according to species, subspecies, varieties, level of ploidy and genotypes.

The three subspecies of O.sativa viz., indica japonica and javanica showed differences in sensitivity to radiations as well as chemical mutagens, as measured by survival, growth

rate and fertility in the M_1 generation. Sensitivity was the highest in javanica followed by japonica and indica. Many investigators have stressed the increased radiosensitivity of japonica varieties in relation to indicas (Matsuo et al., 1958; Nagamatsu and Katayama, 1959; Sin and Huang, 1960; Yamaguchi and Kobayashi, 1960; Fujii, 1962; Kawai, 1962, 1963a; Yamaguchi, 1964; Joshua et al., 1965; Narahari, 1969; Swaminathan et al., 1970; Singh, 1970). However, Swaminathan (1966a), Siddiq (1967) and Siddiq and Swaminathan (1968a) found that the highly mutagen sensitive japonica variety, Taichung-65 had a lower mutation frequency than the indica variety, Taichung native-1. Singh (1970) on the other hand, reported that irrespective of the type and dose of mutagens the japonica varieties showed a higher mutation frequency than the indicas. Since japonicas are relatively more radiosensitive than indicas, the increased mutation frequency in japonicas was stated to be quite possible due to the coincident effects of mutagens on M_1 injury and M_2 chlorophyll mutation frequency.

Marked intervarietal differences in radiosensitivity were recorded by Matsuo et al. (1958), Fujii (1962), Bilquez et al. (1965), Ukai (1967), Mikaelsen and Navaratna (1968) and Miah et al. (1970). Intervarietal differences were reported within japonica by Yamaguchi (1956) and Myttenaere et al. (1965) and within indica by Singh (1970).

Differential radiosensitivity of varieties has been traced to the genotype by Matsuo et al. (1958), Chao and Chai

(1961), Fujii (1962), Miah and Bhatti (1968) and Naraharі (1970a). Shama Rao and Bora (1961) reported that different alleles at the same locus are differentially radiosensitive. Osone (1959) and Ota et al. (1959) found hybrids to be more radioresistant than their parents, whereas Joshua et al. (1966) found hybrids to be more sensitive. Yamaguchi and Kobayashi (1960) observed that the hybrids in reciprocal crosses differed in radiosensitivity and Yamaguchi (1964) stated that in addition to a gene or gene system the cytoplasm may also be involved in intervarietal difference of radiosensitivity.

Radiosensitivity of haploid plants was found to be higher than that of diploid by Tanaka (1970). The diploids in turn were reported to be more sensitive than the respective autotetraploids (Yamaguchi and Ando, 1959; Yamaguchi and Kobayashi, 1960; Yamaguchi, 1964 and Sree Rangasamy, 1970).

Naraharі (1969) could not relate differences in sensitivity of varieties to characters such as duration, grain size and shape, whereas Mikaelsen and Navaratna (1968) found in two sets of experiments that the varieties with the smallest grain were the most radioresistant. Singh (1970) reported that the correlation between nuclear volume measured from pollen and radiosensitivity was not significant. The varietal differences in radiosensitivity were also reported to be due to differences in chemical composition (Myttenaere et al., 1965) or due to differences in endogenous levels of auxin and ascorbic acid (Goud et al., 1967).

MATERIALS AND METHODS

MATERIALS AND METHODS

A. MATERIALS

I. Biological material

Seeds (Caryopsis) of the variety Co.29 of *Oryza sativa* subsp. *indica* were used for the main experiments in the present investigation. This is a short duration variety (seed to seed - 120 days) with tall stature, semi-compact tillers, light green leaves, semi-compact exerted ears and medium fine seeds. It is moderately resistant to the blast disease.

For the study of differences in radiosensitivity, 20 varieties of *Oryza sativa* were selected, the salient features of which are presented in Table I.

II. Mutagens

Three radiations (ionising) viz., gamma rays, X-rays and fast neutrons and five chemical mutagens (monofunctional alkylating agents) viz., diethyl sulphate (DES), ethyl methanesulphonate (EMS), methyl methanesulphonate (MMS), nitroso methyl urea (NMU) and methyl nitro nitroso guanidine (MNNG) were used in the present study.

a. Radiations

Gamma irradiation was done by using a 2000 Ci ^{60}Co gamma cell installed at the Indian Agricultural Research Institute, New Delhi. Six different doses in the range of

TABLE I

Particulars of varieties of rice

Sl. No.	Name of variety	Sub species	Stature of plants	Flowering duration (days)*	Grain size		Weight of 1000 grains (g)
					Length (mm)	Breadth (mm)	
1.	Co.29	indica	Tall	88	8.2	2.8	23.8
2.	Co.10	"	"	88	8.4	3.1	26.0
3.	Co.13	"	"	87	8.2	3.0	25.9
4.	Co.18	"	"	93	8.2	2.9	22.4
5.	Adt.27	"	"	77	6.2	3.0	16.3
6.	Adt.28	"	"	76	8.3	3.3	28.5
7.	Tkm.6	"	"	86	8.3	2.4	18.4
8.	Ptb.10	"	"	78	8.6	3.1	26.3
9.	Ptb.23	"	"	77	8.6	3.0	26.6
10.	Ptb.31	"	"	72	7.6	3.3	26.8
11.	Peta	"	"	112	8.7	3.0	27.3
12.	Norin-1	japonica	Dwarf	78	7.2	3.3	23.5
13.	Norin-6	"	"	65	7.0	3.4	26.4
14.	Norin-17	"	"	69	7.1	3.5	28.0
15.	Nikku-132	"	"	67	6.7	3.4	26.7
16.	Taichung native-I	Dwarf indica	"	95	7.7	3.1	25.1
17.	I.R.8	"	"	102	9.1	3.1	30.1
18.	Tainan-3	Tropical japonica	Tall	94	6.9	3.6	27.1
19.	Taichung-65	"	"	82	7.1	3.5	27.7
20.	Kaohsiung-68	"	"	95	7.0	3.6	28.8

* at Coimbatore

10 to 60 krad were employed. X-irradiation was done by using a Philips X-ray generator operated at 50 kV and 2 mA installed at the Agricultural College and Research Institute, Coimbatore. Twelve different doses in the range of 5 to 40 krad were employed. Fast neutron irradiation was done at the ASTRA swimming-pool-type reactor in the standard neutron irradiation facility (SNIF) described by Burtscher and Casta (1967) with the help of Dr.K.Mikaelsen of the International Atomic Energy Agency, Vienna. Eight different doses in the range of 705 to 2100 rad were employed.

b. Chemical mutagens

The particulars of chemical mutagens and the dose range employed are given in Table II.

B. METHODS

I. Methods of mutagenic treatments

Seeds were collected from a population of plants grown for Breeder's seed at the Paddy Breeding Station, Agricultural College and Research Institute, Coimbatore. Mature ears were harvested and hand threshed to prevent damage due to mechanical injury. Well filled seeds of uniform size were hand picked to obtain the sample for mutagenic treatments. Seeds were uniformly dried and stabilised for moisture content of 10 to 12 per cent. Moisture content was determined by the hot air oven method suggested in the proceedings of the International Seed Testing Association (Anon., 1966a). In all experiments seeds with

TABLE II

Particulars of chemical mutagens

Mutagen	Source	Molecular weight	Density (g/ml)	No. of doses tried	Dose range(mM)
1. Diethyl sulphate $(C_2H_5O)_2\ SO_2$	British Drug House, U.K.	154.19	1.178	5	11.4-57.0
2. Ethyl methanesulphonate $CH_3SO_2OC_2H_5$	Eastman Organic Chemicals, U.S.A.	124.16	1.204*	12	19-384
3. Methyl methanesulphonate $CH_3SO_2OCH_3$	"	110.13	1.298*	13	1.2-29.5
4. Nitroso methyl urea $H_3C-N(NO)-CO-NH_2$	K & K Laboratories, U.S.A.	103.11**	Solid	8	0.97-9.70
5. Methyl Nitro Nitroso guanidine $H_3C-N(NO)-C(=NH)-NHNO_2$	"	147.09**	Solid	8	0.68-10.20

* Leicht, R.J. (1970). Eastman Organic Chemicals, U.S.A. Personal communication

** Kolier, I. (1970) K & K Laboratories, U.S.A. Personal communication

hull were used. In the main experiments, 400 seeds were treated with each dose of every mutagen.

a. Radiations

The gamma source was stationary and irradiation was done at a dose rate of 2500 rad per minute by moving down a cylindrical gasket carrying the seeds. X-irradiation was done in air without filter at a target distance of 4 cm from the source at a dose rate of 500 rad per second. Fast neutron irradiation was done with the SWIF located in position E6 at a distance of 62.5 cm from the core where the dose rate was 1.49 rad per second. The reactor was operated at a power level of 5.0 MW. The gamma radiation and thermal neutron contaminations amounted to 2% and 0.3% respectively, of the total dose given. The absorbed dose for the seeds was computed by the sulphur pellet measurements (Mikaelsen and Brunner, 1968; Mikaelsen, 1968a).

b. Chemical mutagens

The procedure for treatment with chemical mutagens was based on the recommendations of the third FAO/IAEA research coordination meeting on the use of induced mutations in rice breeding (Anon., 1967). Seeds were soaked for 16 hours by submersion in distilled water. The different concentrations of mutagens were prepared in double distilled water. The presoaked seeds were pressed between folds of blotting paper to remove the superficial water and treated by keeping immersed in the mutagen solution for eight hours with

intermittent shaking. The volume of the mutagen solution used was 25 ml per 100 seeds (approximately 10 times the volume of seeds) which facilitates uniform absorption of the mutagen by the seeds. The temperature of the solution was maintained at 73 ± 2°F throughout the period of treatment.

In treating with EMS, MMS, NMU and MNNG, seeds were soaked continuously for eight hours without changing the mutagen solution. But in the case of DES, the duration of treatment was only four hours. The DES solution was changed once in every 40 minutes with freshly prepared solution to maintain the activity of the mutagen at a high and nearly constant level throughout the period of treatment since the half life of DES at 77°F (25°C) is 1.77 hours (Konzak et al., 1961). At the highest concentration of DES viz., 57 mM, a part of the chemical remained as an oily precipitate without going into solution. This is in agreement with the report of Konzak et al. (1961) that the maximum solubility of DES in water is 45 mM at 68°F (20°C). The seeds after treatment were washed in water for one hour.

c. Combination treatments

Two combination treatments of radiations and chemical mutagens were employed. Irradiation was done first. The seeds were then soaked in distilled water and treated with the chemical mutagen.

i) Fast neutrons + NMU: Three doses of fast neutrons viz., 705, 968 and 1170 rad and four doses of NMU viz., 0.97,

1.94, 2.91 and 3.88 mM were employed in all possible combinations.

ii) Gamma rays + NMU: Three doses of gamma rays viz., 10, 20 and 30 krad and two doses of NMU viz., 0.48 and 0.97 mM were employed in all possible combinations.

d. Recurrent and alternate treatments

One fertile primary ear was selected from each of 100 M_1 plants in every one of the doses 10, 20 and 30 krad of gamma rays and from the untreated population to serve as control. Three seeds were selected from each of these ears conforming to the four groups to form a lot of 300 seeds. Thus, seven lots of 300 seeds each were made up in each of the four groups to form the seed material for the second cycle of mutagen treatments. Out of the seven lots in each group, one was left untreated, three were subjected to irradiation at the three doses of gamma rays in the recurrent treatment and the remaining three were treated with three doses of NMU in the alternate treatment. In the recurrent treatment the doses of gamma rays employed were 10, 20 and 30 krad while in the alternate treatment the doses of NMU employed were 0.97, 1.94 and 2.91 mM.

e. Presoaking of seeds before treatment with mutagens

In this experiment there were three series, each corresponding to treatment with one of the three dose-duration combinations of NMU viz., 0.97 mM for four hours, 2.91 mM for two hours and 2.91 mM for four hours. Each series consisted of

11 different periods of presoaking in the range of eight to 48 hours at intervals of four hours, thus, constituting a total of 34 treatments including one control.

Three hundred seeds were subjected to each treatment. Seeds were soaked by complete submersion in distilled water for the appropriate period. In treatments having more than 24 hours presoaking, seeds were submerged in water for 24 hours and kept in covered petridishes lined with wet filter paper for the rest of the period to protect the seeds from drying up and to ensure oxygen availability for metabolic activity of the germinating seeds. Presoaking for different treatments commenced at appropriately different times and all mutagen treatments were carried out simultaneously under identical conditions. The schedule for EMU treatment was the same as that described for chemical mutagens earlier, except in that the treatment in this case was done at room temperature (80 ± 2°F) and was only for periods of two and four hours.

II. Handling of materials after mutagenic treatments

Following mutagen treatment, the material was handled in the immediate and subsequent generations as per the recommendations of the panel meeting on Coordination of research on the use of mutations in rice breeding (Anon., 1966b).

a. M_1 generation

Seeds irradiated with X-rays and treated with chemical mutagens were sown immediately, whereas seeds irradiated with

gamma rays and fast neutrons were sown three and four weeks respectively, after irradiation. They were sown partly in petridishes and partly in a field nursery. Thirty seeds from each treatment were sown in one petridish lined with wet filter paper which was replicated thrice for making counts of germination and for measuring the lengths of coleoptile and primary root. In the field nursery 100 seeds from each treatment were sown in one small plot of size 60 x 40 cm and this was replicated thrice. The irradiated seeds were soaked for 24 hours before sowing, whereas the seeds treated with chemical mutagens were sown as such. Each seed was sown flat on the soil surface, with embryo on the side, at uniform spacing. Thirty days old seedlings were transplanted in the main field in singles at a spacing of 20 x 10 cm. The seedlings were planted close to reduce tillering of M_1 plants, because with excessive tillering there would be a dilution effect of mutations in later formed panicles due to diplontic selection. The following observations were made in the M_1 generation.

i) Germination: Germinated seeds in petridishes were counted daily from the second to the seventh day. Emergence of both primary root and coleoptile was taken as the criterion for germination.

ii) Length of coleoptile and primary root: These measurements were made on seedlings in petridishes. Fifteen germinated seeds from each replication of every treatment were selected at random, after 96 hours from soaking and the lengths measured to the tip of the coleoptile and primary root.

iii) Survival: The surviving seedlings in the nursery were counted on the 15th and 30th days. The final survival count was made at the time of flowering. All plants with green colour (photosynthetic tissue) were counted as surviving.

iv) Plant height: Seedling height was measured on the 8th day on seedlings in petridishes and on the 15th and 30th days on seedlings in the nursery (Figure 1). Height of mature plants was measured in the main field on completion of flowering. Measurements at all stages were made on 15 plants selected at random from each replication of every treatment.

v) Chlorophyll deficient chimeras: The plants in the nursery and the main field were examined periodically for the presence of chlorophyll deficient sectors on leaves. All chimeras for chlorophyll deficiency were scored individually and described. The tillers were marked specifically for the differential sectors.

vi) Harvesting of M_1 plants: At the time of flowering, 90 to 120 plants in each of the dose groups were selected at random. The apical and primary axillary panicles of these plants were labelled and bagged to avoid cross pollination. At maturity they were harvested and stored separately in paper bags.

vii) Fertility: Seed fertility was estimated by counting grain and chaff on both the ears from 60 M_1 plants in each dose.

Figure 1 M_1 seedlings in field nursery.

1 Control

2 to 7 From seeds irradiated with gamma rays at 10, 20, 30, 40, 50 and 60 krad respectively.

Figure 2 Procedure adopted for the study of induced variation for quantitative characters.

Figure 1

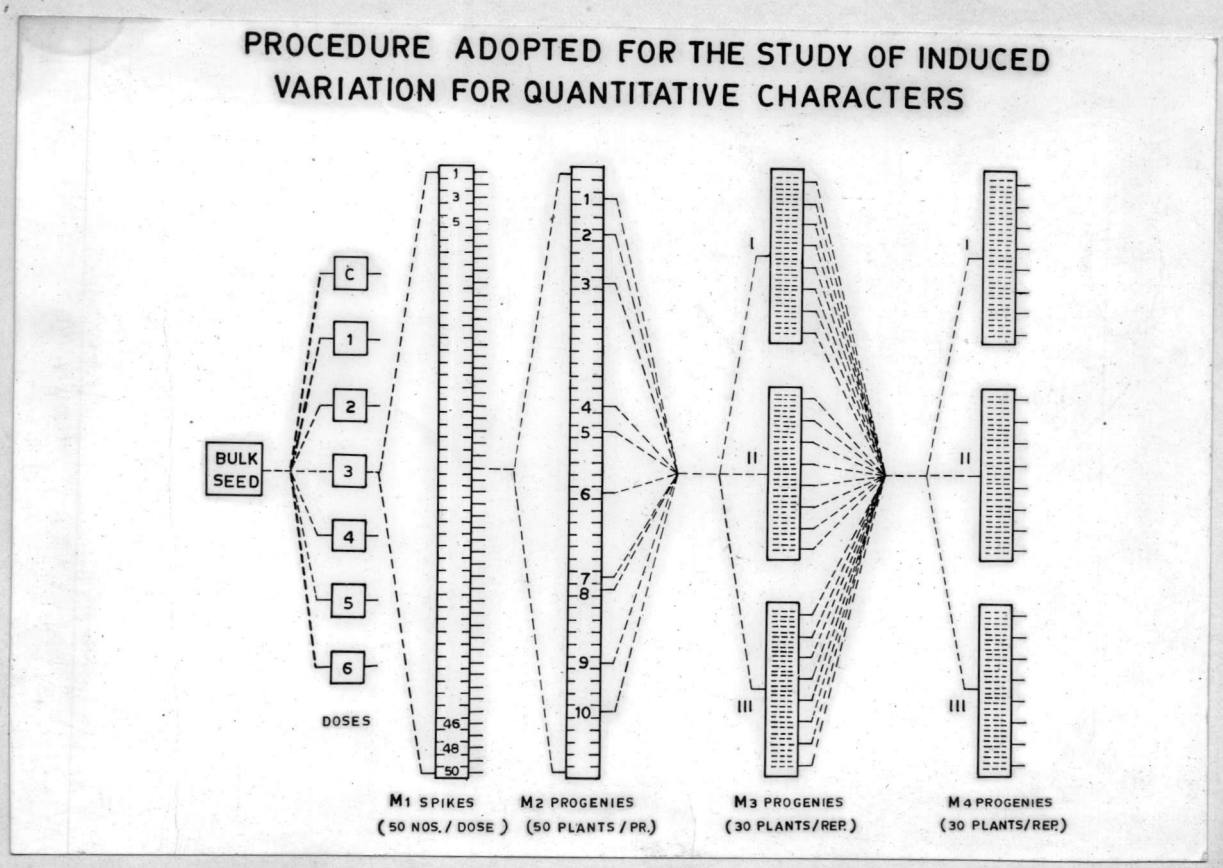

Figure 2

b. M_2 generation

The seeds of M_1 plants were sown in a field nursery. All the seeds from each ear were sown in separate beds. On the 30th day a maximum of 50 seedlings per ear were transplanted in the main field as ear-progeny rows. Single seedlings were planted at a spacing of 20 x 10 cm. The following observations were made in the M_2 generation.

i) Chlorophyll mutations: The nursery beds were examined at intervals of three to five days during the period of eight to 20 days following sowing to spot out chlorophyll deficient seedlings. The ear-progenies segregating for chlorophyll mutants were scored first to calculate mutation frequency per 100 M_1 plants and per 100 M_1 "spikes"* (ears). The total number of mutants and normal seedlings was also counted to estimate the mutant frequency per 100 M_2 plants. In the segregating progenies, the mutants and normal seedlings were counted separately to calculate the segregation ratios (percentage of mutants in mutated ear-progenies). The chlorophyll mutants were classified according to the system suggested by Gustafsson (1940) and expanded by Konzak et al. (1968). The different types were scored separately for calculating the spectrum (relative percentage of different types) of mutants.

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* The inflorescence in rice is a panicle. The term "mutations per 100 M_1 spikes" is being frequently used by several investigators to refer to the estimate of mutation frequency on ear basis in cereals including rice. For uniformity in terminology this term is used herein to mean mutations per 100 M_1 ears.

ii) Abnormal seedling and nonviable mutations: The nursery beds were examined for the presence of abnormal seedling and nonviable mutants. The types present were described and the mutant and normal seedlings in segregating progenies were counted separately.

iii) Viable mutations: This study was confined to four mutagens viz., gamma rays, fast neutrons, EMS and NMU. The M_2 plants were observed periodically during their entire life period and viable mutations were scored on ear-progeny basis. All the visible changes were scored. These mutants were described with respect to deviations from normal plants, labelled and harvested separately. Viable mutation frequency per 100 M_1 spikes was estimated.

iv) Total mutations: The scores for chlorophyll and viable mutations were used for estimating the total mutation frequency. A progeny was counted as segregating, if it segregated for a chlorophyll and/or a viable mutation. Total mutation frequency was estimated as number of mutations per 100 M_1 spikes.

v) Estimation of mutagenic effectiveness and efficiency: The effectiveness and efficiency of mutagens in inducing chlorophyll and viable mutations were estimated adopting the formulae suggested by Konsak et al. (1965a).

Mutagenic effectiveness - M/to or krad,

Mutagenic efficiency - M/L; M/I and M/S,

where,

M - Chlorophyll mutations per 100 M_1 spikes,

t - period of chemical mutagen treatment in hours,

c - concentration of chemical mutagens in millimoles,

krad - dose of radiations in kilorad,

L - % lethality i.e., survival reduction of seedlings at 30 days,

I - % injury i.e., height reduction of seedlings at 30 days, and

S - % sterility i.e., reduction in seed fertility.

vi) Estimation of relative biological effectiveness (RBE): The RBE of gamma rays and fast neutrons was estimated with X-rays as the standard radiation. The dose of X-rays was divided by the doses of gamma rays and fast neutrons producing the same effect to calculate the RBE values for the several criteria.

vii) M_1 chlorophyll chimeras and M_2 chlorophyll mutation frequency: Panicles on chimeric and normal tillers of M_1 plants with chlorophyll deficient sectors in treatments with fast neutrons, EMS, NMU, and fast neutrons in combination with NMU were labelled and on maturity harvested separately. They were sown on ear-progeny basis and scored for the frequency and type of M_2 chlorophyll mutations.

viii) M_1 fertility and M_2 chlorophyll mutation frequency: Mature ears of M_1 plants following seed treatment with different doses of gamma rays and EMS were harvested. Based on the

percentage of seed fertility they were grouped into five classes viz., more than 80, 61 to 80, 41 to 60, 21 to 40 and less than 20. Chlorophyll mutation frequency was estimated on M_1 spike basis and M_2 seedling basis in the different fertility classes separately in each dose. The various types of chlorophyll mutants were also scored separately.

III. Experiments for enhancing mutagenic efficiency

a. Combination treatments

The treated seeds were sown in a field nursery in three replications. Seedling survival counts and height measurements were made on the 15th and 30th days. In the combination of fast neutrons with NMU, the first five panicles in the order of emergence were labelled in 80 M_1 plants per dose combination and harvested separately on maturity. In the combination of gamma rays with NMU only the first two panicles were selected. The M_2 generation was raised on ear-progeny basis and chlorophyll mutation frequencies per 100 M_1 spikes and 100 M_2 plants were estimated.

b. Recurrent and alternate treatments

Seeds after the second cycle of mutagen treatment were sown in the nursery in three replications to raise the second $(M_2^2)^*$ generation. Seedling survival counts and height measurements were made on the 15th and 30th days. The main

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* The superscript refers to the number of mutagenic treatments applied to the seeds in subsequent generations and the subscript refers to the number of generations.

panicles of all surviving plants were labelled and ears harvested separately. The third (M_3^2) generation was raised on ear-progeny basis. Chlorophyll mutations were scored and frequencies per 100 M_2^2 spikes and per 100 M_3^2 plants were estimated.

c. Presoaking of seeds before treatment with mutagens

The treated seeds were sown in the nursery in three replications. Survival count and height measurement were made on the 30th day. Chimeras for chlorophyll deficiency were scored after transplanting and a final survival count was made on completion of flowering. The first five panicles in the order of emergence were labelled in 100 plants selected at random, in each of the 22 treatments belonging to the first two series and the control. The third series of 11 treatments which received a dose of 2.91 mM of EMU for four hours were not advanced to the M_2 generation, since the number of surviving M_1 plants in certain of the treatments were very small. The M_2 generation was raised on ear-progeny basis. Chlorophyll mutation frequencies were estimated as mutations per 100 M_1 plants and 100 M_1 spikes. The different types of mutants were scored separately to estimate the spectrum of mutants. The segregation ratios of mutants were also estimated.

IV. The study of mutated sectors

M_1 ear-progenies segregating with a strong deficit of chlorophyll and other mutants in the M_2 generation were selected for the study of mutated sectors. The M_2 segregation

ratios for each of such progenies were calculated. A number of normal M_2 plants were selected in each progeny. Seeds from these plants were collected separately and plant progenies raised in the M_3 generation. The M_3 progenies from heterozygous M_2 plants were counted and classified. The M_3 segregation ratios for each mutation were estimated.

A significant difference between the M_2 and M_3 segregation ratios relating to the same mutation was taken as an indication of mutated sector in the M_1 ear. M_2 progenies with deficit of recessives and a corresponding deficit of heterozygotes were utilised for the estimation of the size of the mutated sector. The size of the sector was calculated by dividing the M_2 segregation ratio by the corresponding M_3 ratio. The number of initial cells relating to the M_1 ear was taken as the reciprocal of the sector size.

V. Study of induced variation in quantitative characters

This study was made in the M_2, M_3 and M_4 generations following treatments with gamma rays at doses 10, 20 and 30 krad and EMS at doses 38, 77 and 115 mM. Fifty ear-progenies were selected in the M_2 generation in each of the six doses and from the untreated control population. Progenies segregating for chlorophyll mutations, other lethal mutations and sterility were not selected. Plants with conspicuous morphological differences (viable mutants) were also not included. Thus, all plants showing recognisable changes were eliminated from the population. The progenies were carried forward separately

from M_2 to M_4 generations. The procedure adopted is schematically presented in Figure 2. The characters studied were duration, height of plants, number of ears, length of ear and number of spikelets per panicle.

a. M_2 generation

Fifty progenies in each of the six doses and the control were grown in progeny rows 20 cm apart, in singles, at a spacing of 10 cm within the row. At the time of flowering, 10 plants per progeny were selected at random from among the apparently normal plants and labelled. Plants bordering on a gap and at margins were not selected. Observations on the first three characters were made on these labelled plants and the last two characters were studied on their main panicles. Seeds from the 10 plants in each progeny were bulked.

b. M_3 generation

The bulk seeds in each M_2 progeny were used to raise the M_3 family rows. The 350 families were grown in three replications with three rows of 10 single plants in each replication of every family. The eight middle plants in the central row in each replication of every family were selected for collecting data on the quantitative characters. Seeds from the 24 selected plants in each family were bulked.

c. M_4 generation

The bulk seeds of M_3 families were used to raise the

M_4 families. The field design and methods for collection of data were the same as in the M_3 generation.

VI. Radiosensitivity of varieties

Seeds of 20 varieties of rice listed in Table I were carefully selected and stabilised for moisture content of 10 to 12 per cent by keeping them in a desiccator over a saturated solution of calcium chloride for a period of four weeks (Kawai and Sato, 1966). One hundred seeds in each variety were treated with each of the six doses of X-rays viz., 5.0, 7.5, 10.0, 12.5, 15.0 and 20.0 krad.

Seeds were sown in a field nursery in two replications and seedling height was measured on the 15th and 30th days and survival count made on the 30th day. The main panicles of all the surviving plants were labelled and harvested. Seed fertility was estimated.

The two higher doses viz., 15 and 20 krad were carried over to the M_2 generation and chlorophyll mutations were scored. The frequency was estimated as mutants per 100 M_2 plants. The different types of mutants were scored separately and the mutation spectrum was estimated.

VII. Abbreviations

krad : Kilo rad

mM : Millimoles

LD_{50} : Dose causing 50% reduction

M_1 : First generation after mutagen treatment

M_2 : Second generation after mutagen treatment

M_3 : Third generation after mutagen treatment

M_4 : Fourth generation after mutagen treatment

M_2^2 : Second generation in repeated mutagen treatments

M_3^2 : Third generation in repeated mutagen treatments

Chlorophyll mutants

A : Albina

X : Xantha

V : Viridis

C : Chlorina

AV : Alboviridis

VA : Viridoalbina

XA : Xanthalba

S : Striata

T : Tigrina

M : Maculata

BL : Brown lamina

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

I. Effects of mutagens in the M_1 generation

The percentages of germination recorded on the 4th and 7th days are presented in Table III. Germination was not affected by radiations even at very high doses, whereas the percentages decreased with increasing doses of chemical mutagens (Figure 3). The percentage of germination on the 7th day was more than that on the 4th day in treatment with chemical mutagens and gamma rays indicating a delay in germination. A retardation in germination was evident by the increase in the mean period with increasing doses. This effect was pronounced in treatments with chemical mutagens such as NMU and MNNG.

The percentages of survival estimated on the 15th day, 30th day and at maturity are given in Table III. There was a decrease in survival with increasing doses of the mutagens (Figure 3). Gamma rays among radiations and NMU and MNNG among the chemical mutagens led to considerable reduction in plant survival at higher doses. A further reduction in survival at the advanced stages of growth was recorded at the higher doses of gamma rays and X-rays. On the other hand, in treatment with chemical mutagens such as NMU and MNNG survival reduction due to lethality of plants occurred mostly at the early seedling stage.

The mean lengths of coleoptile and primary root measured after 96 hours from the time of soaking are presented in

TABLE III

Effect of mutagens on germination of seeds and survival of plants in the M_1 generation

Mutagen and dose	Germination (%)		Mean period for germination		Survival (% of control)		
	4th day	7th day	Days	% of control	15th day	30th day	At flowering
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i) Gamma rays							
Control	100	100	2.70	100	100	100	100
10 krad	100	100	2.96	109	100	100	100
20 ,,	98	100	3.41	126	102	101	99
30 ,,	91	100	3.53	131	98	97	92
40 ,,	80	100	3.73	138	79	63	47
50 ,,	88	100	3.70	137	37	21	13
60 ,,	79	100	3.98	147	2	0	0
ii) X-rays							
Control	100	100	2.88	100	100	100	100
5.0 krad	100	100	3.02	105	101	101	98
7.5 ,,	100	100	3.06	106	102	101	96
10.0 ,,	100	100	3.08	107	101	101	98
12.5 ,,	100	100	3.02	105	103	102	95
15.0 ,,	100	100	3.04	106	100	100	96
17.5 ,,	98	100	3.12	108	102	103	92
20.0 ,,	100	100	3.12	108	97	97	86
22.5 ,,	100	100	3.00	104	100	99	90
25.0 ,,	98	100	3.14	109	97	97	81
30.0 ,,	100	100	3.12	108	99	98	72
35.0 ,,	98	100	3.16	110	98	93	68
40.0 ,,	96	98	3.24	112	84	74	58
iii) Fast neutrons							
Control	100	100	3.05	100	100	100	100
705 rad	99	100	3.12	102	100	100	100
968 ,,	100	100	3.07	101	99	98	97
1170 ,,	99	100	3.09	101	99	98	97
1408 ,,	99	100	3.13	102	101	99	98
1570 ,,	100	100	3.08	101	98	98	96
1710 ,,	100	100	3.11	102	99	99	96
1880 ,,	100	100	3.09	101	100	99	97
2100 ,,	100	100	3.12	102	99	98	95
iv) DES							
Control	100	100	2.71	100	100	100	100
11.4 mM	100	100	3.05	113	93	93	94
22.8 ,,	86	93	3.32	123	86	87	88
34.2 ,,	73	78	3.27	121	77	79	79
45.6 ,,	72	75	3.18	117	71	70	73
57.0 ,,	73	76	3.22	119	71	71	72

TABLE III (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
		✓		✓		✓	
v) EMS							
Control	100	100	2.91	100	100	100	100
19 mM	100	100	3.00	103	101	100	98
38 "	100	100	3.00	103	97	97	90
✓ 58 "	100	100	3.03	104	98	96	90
77 "	100	100	3.23	111	98	97	90
96 "	95	100	3.43	118	96	94	89
✓ 115 "	89	100	3.53	121	91	89	88
154 "	84	100	3.51	121	91	88	88
✓ 192 "	86	92	3.47	119	90	89	88
✓ 240 "	84	88	3.57	123	89	88	87
✓ 288 "	82	88	3.65	125	90	89	85
336 "	86	90	3.64	125	86	85	83
384 "	78	86	3.90	134	84	83	82
vi) MMS							
Control	100	100	2.91	100	100	100	100
1.2 mM	100	100	2.85	98	99	96	95
2.4 "	100	100	2.87	99	98	98	97
3.6 "	100	100	2.89	99	102	99	96
4.8 "	99	100	2.90	100	101	99	98
5.9 "	100	100	2.91	100	101	97	95
7.1 "	93	100	3.22	111	98	98	94
8.3 "	88	96	3.38	116	98	98	97
9.5 "	88	94	3.42	118	88	87	86
11.8 "	84	90	3.39	116	83	82	81
14.2 "	82	90	3.43	118	73	72	70
17.7 "	76	86	3.88	133	76	73	71
23.6 "	78	86	3.94	135	71	72	71
29.5 "	68	84	4.16	143	69	70	70
vii) NMU							
Control	100	100	2.82	100	100	100	100
0.97 mM	100	100	2.94	104	97	98	98
✓ 1.94 "	100	100	3.14	111	85	79	78
2.91 "	92	100	3.58	127	64	53	48
✓ 3.88 "	87	99	3.93	139	54	46	45
4.85 "	91	100	4.08	145	49	44	39
✓ 5.82 "	87	100	4.13	146	40	35	32
✓ 7.76 "	85	94	4.40	156	29	24	20
✓ 9.70 "	66	82	4.75	168	25	22	10
viii) MNNG							
Control	100	100	2.51	100	100	100	100
0.68 mM	100	100	2.82	112	101	100	98
1.36 "	100	100	2.85	114	100	98	95
2.72 "	100	100	3.03	121	98	98	94
4.08 "	100	100	3.41	136	94	92	90
5.44 "	100	100	3.94	157	80	79	78
6.80 "	94	100	4.20	167	67	66	63
8.16 "	84	95	4.37	174	50	48	45
10.20 "	73	83	4.65	185	37	35	33

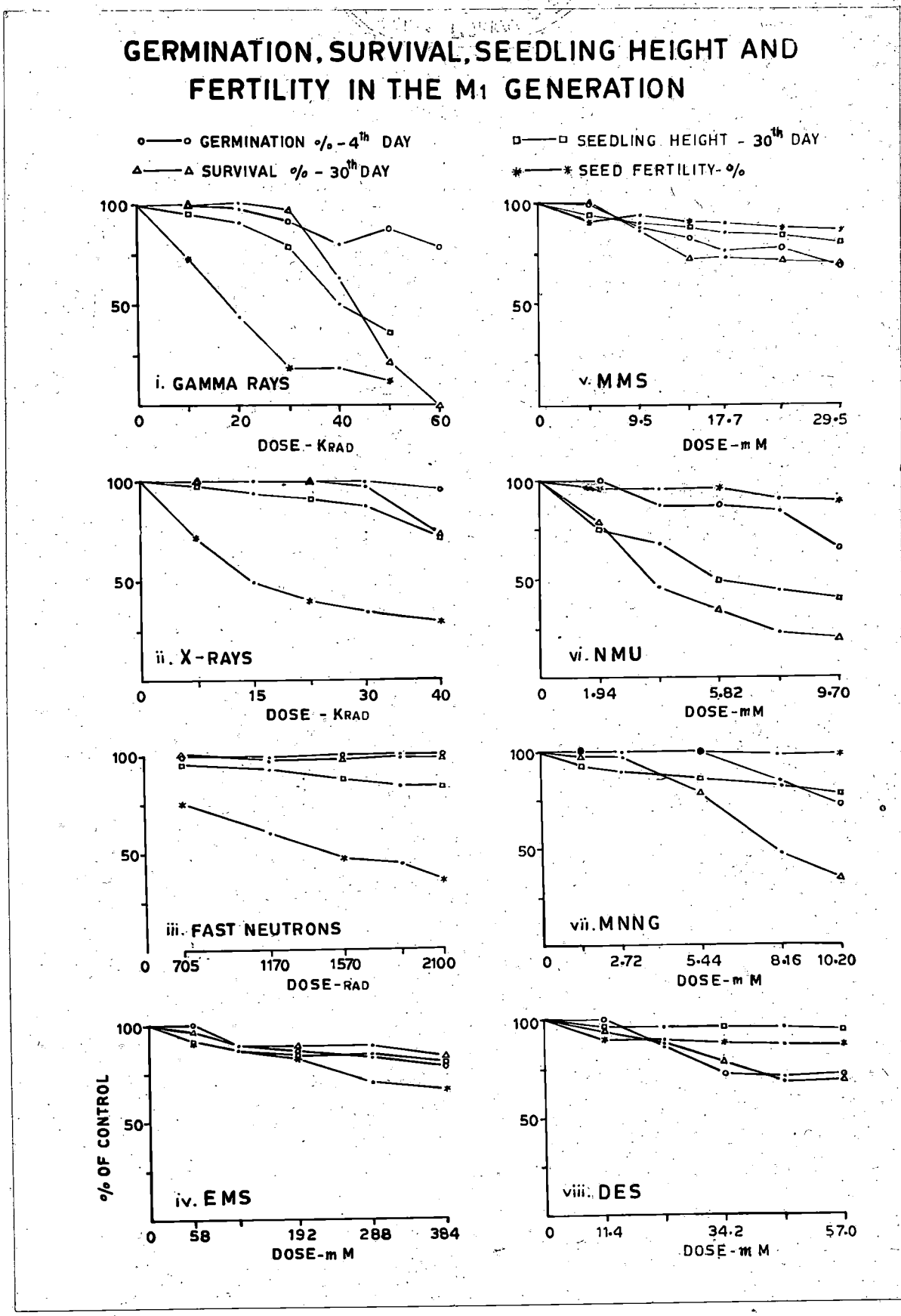

GERMINATION, SURVIVAL, SEEDLING HEIGHT AND FERTILITY IN THE M1 GENERATION
GERMINATION %-4th DAY
SURVIVAL %-30th DAY
SEEDLING HEIGHT-30th DAY
SEED FERTILITY-%
i. GAMMA RAYS
DOSE-KRAD
ii. X-RAYS
DOSE-KRAD
iii. FAST NEUTRONS
DOSE-RAD
iv. EMS
% OF CONTROL
DOSE-m M
v. MMS
DOSE-m M
vi. NMU
DOSE-mM
vii. MNNG
DOSE-m M
viii. DES
DOSE-m M

Figure 3

Table IV. The length of coleoptile and primary roots decreased with increasing doses of radiations as well as chemical mutagens. All mutagens except MNNG inhibited the growth of root more pronouncedly than that of the shoot, whereas MNNG had strongly inhibited the growth of shoot rather than that of root. Gamma rays among radiations and NMU among chemical mutagens were found to induce the maximum inhibitory effect on root growth. The shoot/root ratio increased with an increase in the dose of the mutagens (Table IV), thereby indicating that the damaging effect of mutagens on the root increased with the dose at a faster rate than that on the shoot. The shoot/root ratio was the highest following treatment with gamma rays and NMU at the higher doses.

The mean values for seedling height measured on the 8th, 15th and 30th days and mature plant height at flowering time were converted into percentages of control and presented in Table IV. Plant height was found to decrease with increasing doses of all mutagens except EMS. The effect was drastic with gamma rays among radiations and NMU among chemical mutagens (Figure 3). The magnitude of reduction in height (injury) was the maximum at the early seedling stage (8 to 15 days) and minimum at the stage of maturity for the same dose level indicating that the rate of growth was high at the late stages. This was true in the case of radiations as well as chemical mutagens at all dose levels. The recovery effect was highly conspicuous at the higher doses (Figure 4).

TABLE IV

Effect of mutagens on plant growth in the M_1 generation

Mutagen and dose	Percentage of control						
	Coleoptile length	Primary root length	Coleoptile/ primary root	Plant height			
				8th day	15th day	30th day	At flowering
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
i) Gamma rays							
Control	100	100	100	100	100	100	100
10 krad	96	96	100	96	101	95	96
20 "	96	83	114	87	87	91	92
30 "	79	52	151	70	67	79	84
40 "	65	38	177	37	39	51	80
50 "	63	32	195	23	21	36	74
60 "	56	25	223	13	16	..	..
ii) X-rays							
Control	100	100	100	100	100	100	100
5.0 krad	99	85	118	100	102	101	98
7.5 "	96	80	122	99	96	99	96
10.0 "	97	77	126	96	92	95	96
12.5 "	94	74	126	93	85	94	95
15.0 "	93	76	122	90	84	94	97
17.5 "	87	68	130	85	82	94	94
20.0 "	84	63	133	84	81	92	95
22.5 "	85	59	144	76	76	91	95
25.0 "	76	52	148	76	73	90	94
30.0 "	73	44	166	68	68	87	92
35.0 "	69	40	174	53	64	81	90
40.0 "	58	34	174	41	51	73	86
iii) Fast neutrons							
Control	100	100	100	100	100	100	100
705 rad	96	95	100	95	93	95	98
968 "	94	91	103	91	88	93	96
1170 "	90	90	100	91	87	92	95
1408 "	89	89	100	90	81	90	96
1570 "	88	87	101	89	78	87	92
1710 "	87	76	112	89	76	84	91
1880 "	86	75	114	86	72	83	92
2100 "	84	74	113	85	67	83	91
iv) DES							
Control	100	100	100	100	100	100	100
11.4 mM	93	91	102	96	96	96	95
22.8 "	91	86	106	95	96	96	97
34.2 "	86	84	103	92	95	97	98
45.6 "	89	79	114	91	94	96	99
57.0 "	84	75	112	88	94	95	96

TABLE IV (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
v) EMS							
Control	100	100	100	100	100	100	100
19 mM	102	103	100	98	100	96	98
38 "	89	93	96	98	95	95	96
58 "	89	81	110	88	96	91	94
77 "	79	77	103	77	91	91	95
96 "	73	75	98	71	89	86	92
115 "	71	67	106	70	86	87	94
154 "	72	59	123	72	75	85	91
192 "	70	45	156	69	65	85	94
240 "	68	44	154	60	62	84	90
288 "	67	43	156	55	57	83	92
336 "	61	40	154	52	60	84	94
384 "	51	38	137	49	56	81	90
vi) MMS							
Control	100	100	100	100	100	100	100
1.2 mM	95	99	96	99	98	99	98
2.4 "	98	98	100	101	94	95	96
3.6 "	95	94	101	100	90	92	95
4.8 "	95	95	100	98	90	93	96
5.9 "	97	95	102	93	90	91	94
7.1 "	95	90	106	92	87	91	95
8.3 "	95	75	127	83	87	91	95
9.5 "	94	67	142	81	86	89	92
11.8 "	94	59	160	80	82	88	94
14.2 "	93	55	168	78	70	87	93
17.7 "	79	49	162	67	66	85	94
23.6 "	69	41	169	68	67	84	92
29.5 "	62	32	194	59	62	80	91
vii) NMU							
Control	100	100	100	100	100	100	100
0.97 mM	93	78	117	91	82	88	92
1.94 "	85	53	158	66	76	76	90
2.91 "	79	47	167	56	75	71	86
3.88 "	72	38	188	48	69	68	82
4.85 "	67	31	215	43	56	57	80
5.82 "	62	30	205	40	54	50	82
7.76 "	58	27	213	36	48	45	74
9.70 "	48	21	226	24	38	41	71
viii) MNNG							
Control	100	100	100	100	100	100	100
0.68 mM	91	75	122	90	97	97	99
1.36 "	83	60	137	86	87	93	96
2.72 "	77	47	161	81	83	91	95
4.08 "	68	40	169	68	79	89	94
5.44 "	60	43	139	59	78	86	93
6.80 "	53	49	109	51	77	85	94
8.16 "	44	58	75	44	73	83	92
10.20 "	38	54	69	40	67	79	90

Figure 4 Effect of mutagens on seedlings growth in the M_1 generation

i) Gamma rays

1. 20 krad
2. 30 ,,
3. 40 ,,
4. 50 ,,

ii) X-rays

1. 15.0 krad
2. 22.5 ,,
3. 35.0 ,,
4. 40.0 ,,

iii) Fast neutrons

1. 705 rad
2. 1170 ,,
3. 1570 ,,
4. 2100 ,,

iv) EMS

1. 58 mM
2. 115 ,,
3. 192 ,,
4. 384 ,,

v) NMU

1. 1.94 mM
2. 3.88 ,,
3. 7.76 ,,
4. 9.70 ,,

vi) NNNG

1. 2.72 mM
2. 4.08 ,,
3. 6.80 ,,
4. 10.20 ,,

EFFECT OF MUTAGENS ON SEEDLING GROWTH IN THE M_1 GENERATION

GRAPHS 1-4 FOR DIFFERENT DOSES OF THE MUTAGEN

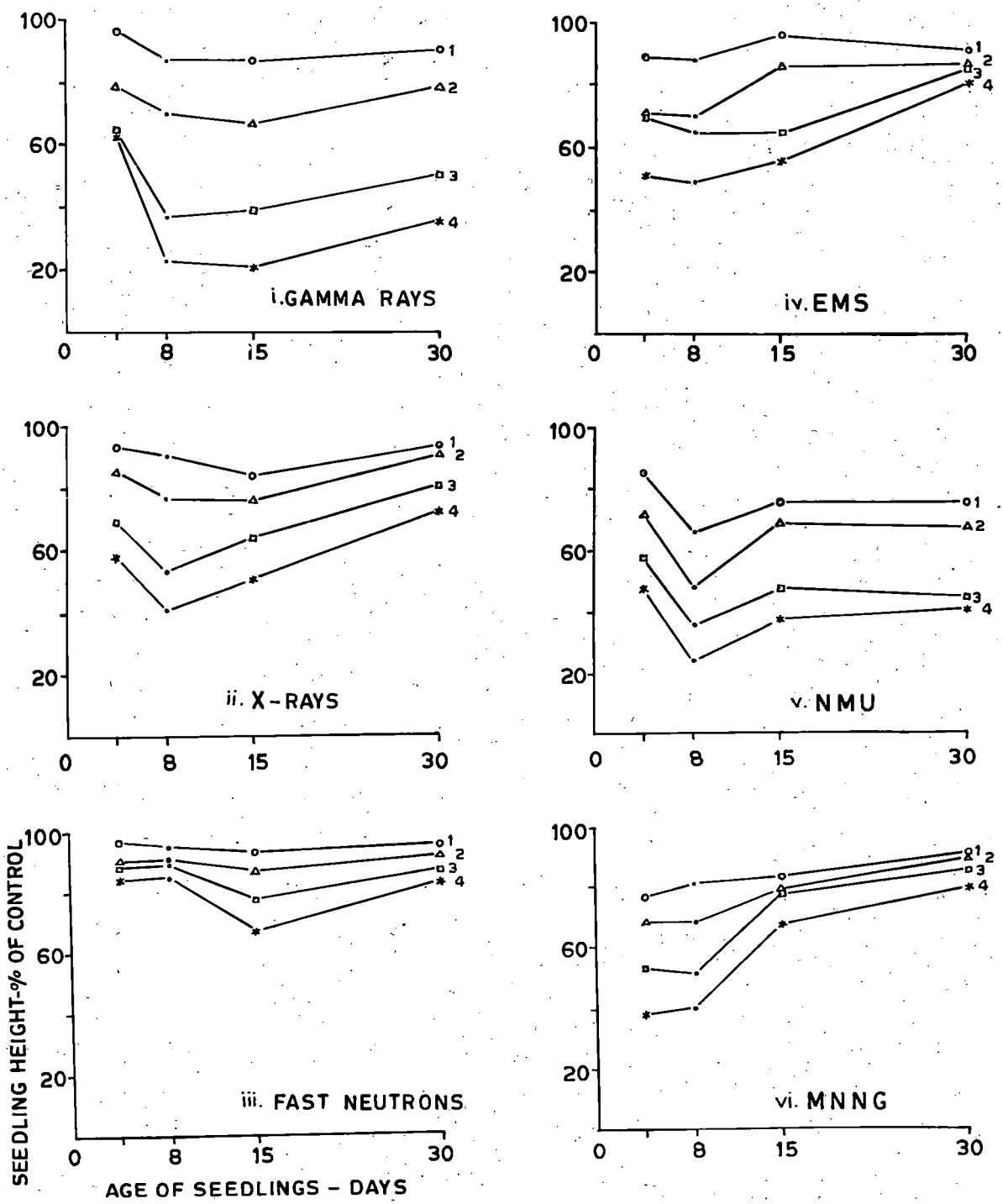


Figure 4

The mean number of spikelets per panicle, an index of the reproductive potential of the M_1 plants, was not significantly effected by the mutagens (Table V). The apparent increase at the high doses of gamma rays might be due to the increased vigour of the plants resulting from low competition for nutrients and water consequent on wider spacing due to high seedling lethality.

The percentages of seed fertility were found to decrease with increasing doses of the mutagens (Table V). The decrease in fertility was more drastic following treatment with radiations than with chemical mutagens. Among radiations the densely ionising fast neutrons was the most effective per unit dose (Figure 3).

The three main estimates of the effects of mutagens viz., lethality, injury and sterility made in the M_1 generation did not show similar trends with increasing doses. Radiations induced more of sterility than lethality and injury at all doses. Chemical mutagens such as EMS, MMS and DES induced lethality, injury and sterility at almost equal intensities; whereas, NMU and MNNG induced more of lethality and injury than sterility (Figure 3). The differential effects of mutagens on the different criteria of damage in the M_1 generation might be traceable to differences in their mode of action and in the induction of lethal effects in the biological system.

Plants with chlorophyll deficient sectors appeared only in treatments with fast neutrons, EMS and NMU, and their

TABLE V

Effect of mutagens on spikelet number and seed fertility in the M_1 generation

Mutagen and dose	No. of panicles studied	No.of spikelets per panicle		Seed fertility	
		Mean	% of control	%	% of control
(1)	(2)	(3)	(4)	(5)	(6)
i) Gamma rays					
Control	60	77	100	95.6	100.0
10 krad	143	72	94	69.9	73.1
20 "	125	83	108	40.8	42.7
30 "	134	78	101	18.2	19.0
40 "	117	99	128	18.7	19.5
50 "	29	115	149	11.6	12.2
ii) X-rays					
Control	60	108	100	95.4	100.0
5.0 krad	114	112	104	70.5	73.9
7.5 "	116	107	99	68.0	71.3
10.0 "	120	105	97	63.0	66.0
12.5 "	120	105	97	58.2	61.0
15.0 "	120	104	96	47.0	49.3
17.5 "	120	109	101	46.5	48.8
20.0 "	120	108	100	43.8	45.9
22.5 "	120	107	99	38.1	40.0
25.0 "	120	110	102	35.6	37.3
30.0 "	120	106	98	34.8	36.5
35.0 "	120	109	103	34.1	35.8
40.0 "	146	113	105	28.5	29.9
iii) Fast neutrons					
Control	60	76	100	96.2	100.0
705 rad	119	74	97	71.8	74.6
968 "	120	77	101	55.5	57.5
1170 "	120	75	99	58.1	60.4
1408 "	119	77	101	53.1	55.2
1570 "	119	75	99	44.7	46.5
1710 "	116	78	103	45.0	46.8
1880 "	119	76	100	42.6	44.3
2100 "	119	83	109	34.8	36.2
iv) DES					
Control	60	89	100	94.7	100.0
11.4 mM	135	89	100	85.3	90.1
22.8 "	136	86	97	84.3	89.1
34.2 "	139	87	98	83.6	88.3
45.6 "	134	94	106	83.1	87.8
57.0 "	139	84	94	83.0	87.6

TABLE V (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)
v) EMS					
Control	60	106	100	96.0	100.0
19 mM	119	104	98	89.7	93.5
38 ,,	119	100	94	88.6	92.3
58 ,,	119	93	88	86.3	90.0
77 ,,	119	102	96	86.7	90.3
96 ,,	117	100	94	84.5	88.0
115 ,,	120	105	99	84.8	88.3
154 ,,	118	81	76	83.1	86.6
192 ,,	120	85	80	79.5	82.7
240 ,,	119	90	85	68.4	71.2
288 ,,	118	85	80	66.8	69.6
336 ,,	206	104	98	66.5	69.3
384 ,,	220	93	88	63.5	66.1
vi) MMS					
Control	60	108	100	94.6	100.0
1.2 mM	116	111	103	86.2	91.1
2.4 ,,	117	107	99	87.2	92.1
3.6 ,,	118	114	106	87.9	93.0
4.8 ,,	120	103	95	86.2	91.1
5.9 ,,	120	107	99	87.7	92.7
7.1 ,,	119	111	103	89.5	94.9
8.3 ,,	119	85	79	87.9	93.0
9.5 ,,	120	84	78	87.6	92.5
11.8 ,,	120	87	81	83.4	88.2
14.2 ,,	120	90	83	85.6	90.5
17.7 ,,	119	82	76	85.8	90.7
23.6 ,,	200	96	89	82.4	87.1
29.5 ,,	199	96	89	82.1	86.8
vii) NMU					
Control	40	89	100	96.8	100.0
0.97 mM	119	78	88	91.3	94.2
1.94 ,,	120	84	94	92.9	96.0
2.91 ,,	118	87	98	92.3	95.5
3.88 ,,	119	84	94	92.9	96.0
4.85 ,,	118	90	101	93.0	96.2
5.82 ,,	120	87	98	92.6	95.8
7.76 ,,	112	91	102	89.1	92.0
9.70 ,,	53	92	103	87.4	90.3
viii) MNNG					
Control	40	85	100	96.3	100.0
0.68 mM	120	81	95	94.6	98.3
1.36 ,,	120	77	91	95.1	98.8
2.72 ,,	119	80	94	95.9	99.6
4.08 ,,	119	82	96	96.0	99.7
5.44 ,,	117	81	95	95.3	98.9
6.80 ,,	119	76	90	94.4	98.0
8.16 ,,	120	78	92	95.3	99.0
10.20 ,,	116	82	96	95.1	98.8

frequencies were found to increase with increasing doses of the mutagens (Table VI). The highest frequencies were obtained in treatment with NMU. Chlorophyll chimeras were not induced by sparsely ionising radiations such as gamma rays and X-rays and by such chemical mutagens as DES, MMS and MNNG. The M_1 chlorophyll chimeras were found to differ considerably with respect to the distribution of chlorophyll deficient sectors. Plants which had one or several tillers with chlorophyll deficient sectors were observed. The sectors were always longitudinal with white, yellow, yellow green or light green colour. They were more prominent on the lamina than on the leaf sheath and culm. The number and width of sectors differed from tiller to tiller and also between leaves of the same tiller. There were several narrow sectors or a few broad ones on each leaf. They were found to arise in the seedling stage or later. The sectors had either faded off or increased in intensity in subsequent leaves. In treatment with NMU, the intensity of striping in several chimeras was so much pronounced as to render the boot leaf in them highly deficient in chlorophyll. In a few cases the sectors extended even to the panicles.

Induced seedling abnormalities were frequent following irradiation at the higher doses. These abnormal seedlings got either eliminated through lethality or recovered and grew into normal plants. An M_1 plant with striking morphological differences induced with gamma rays had narrow leaves and depressed palea and was completely sterile (Figure 5).

TABLE VI

Frequency of chimeras for chlorophyll deficiency in the M_1 generation

Mutagen and dose	No.of M_1 plants scored	No.of chlorophyll chimeras	Percentage of chimeras
1) Fast neutrons			
Control	291	0	..
705 rad	290	0	..
968 "	281	2	0.71
1170 "	282	1	0.35
1408 "	285	2	0.70
1570 "	279	0	..
1710 "	280	2	0.71
1880 "	282	2	0.71
2100 "	277	2	0.72
11) EMS			
Control	355	0	..
19 mM	347	0	..
38 "	319	0	..
58 "	319	1	0.31
77 "	320	2	0.63
96 "	317	2	0.63
115 "	307	6	1.95
154 "	309	8	2.62
192 "	306	5	1.63
240 "	304	8	2.63
288 "	298	6	2.01
336 "	291	8	2.75
384 "	287	10	3.48
111) NMU			
Control	350	0	..
0.97 mM	345	5	1.46
1.94 "	259	11	4.25
2.91 "	169	6	3.55
3.88 "	157	10	6.37
4.85 "	138	13	9.42
5.82 "	114	7	6.14
7.76 "	69	7	10.15
9.70 "	35	7	20.00

Figure 5. Morphological deviant in the M_1 generation induced by gamma rays

1. Morphological deviant

2. Control

Figure 6. Induced ear-chimeras in the M_1 generation showing longitudinal sectors.

C - Control

1 to 3 - Sector for sterility

4 - Sector for abnormal spikelets

5 and 6 Sector for small grains

7 - Sector for awned long grains

Figure 5

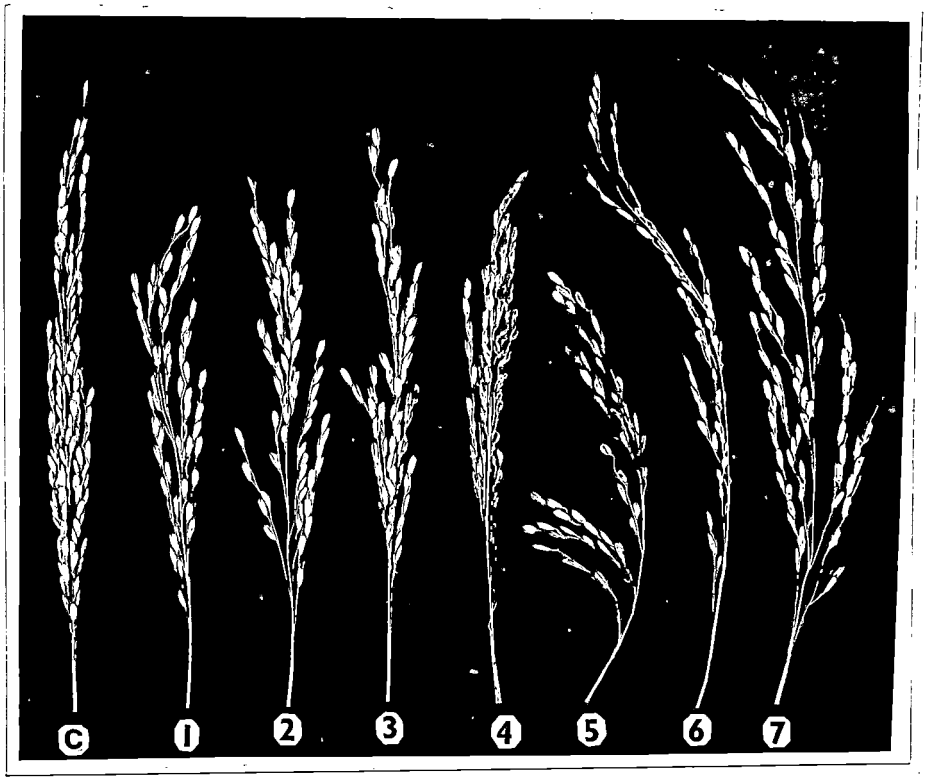


Figure 6

The nature of cytogenetic changes induced by the mutagen resulting in the development of this morphological deviant could not be studied because of abnormal spikelet development.

Chimeras with morphologically different sectors on M_1 ears were observed in a few cases. Such sectors consisted of sterile spikelets, abnormal spikelets, small grains and awned large grains (Figure 6).

II. Studies in the M_2 generation

a. Chlorophyll mutations

Chlorophyll mutations were scored at the seedling stage in the field nursery and their frequencies estimated as the number of mutations per 100 M_1 plants, number of mutations per 100 M_1 spikes and number of mutants per 100 M_2 plants are presented in Table VII. The frequencies increased with an increase in the dose of radiations as well as chemical mutagens and the dose frequency relationship was exponential (Figure 7) upto the middle doses. At the highest dose of relatively more potent mutagens such as gamma rays, fast neutrons, EMS and NMU a drop in mutation frequency was observed. The magnitude of this reduction was low when the frequency was estimated as the number of mutants per 100 M_2 plants. The frequencies were high in treatment with radiations, EMS and NMU.

The relative percentages of different types (spectrum) of mutants are presented in Table VIII and graphically represented in Figure 8. Albins was most frequent following

TABLE VII

Frequency of Chlorophyll mutations in the M_2 generation

Mutagen and dose	No. of M_1 plant progenies		No. of M_1 ear progenies		No. of M_2		Mutation frequency		
	Scored	Segregating	Scored	Segregating	Seedlings scored	Chlorophyll mutants	Per 100 M_1 plants	Per 100 M_1 spikes (ears)	Per 100 M_2 seedlings
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i) Gamma rays									
Control	90	0	180	0	12583	0	..	..	..
10 krad	101	18	195	22	9051	77	17.8	11.3	0.85
20 ,,	97	14	174	14	5365	64	14.4	8.0	1.19
30 ,,	78	7	132	9	2682	32	9.0	6.8	1.19
40 ,,	67	17	111	27	2544	135	25.4	24.3	5.31
50 ,,	21	2	36	2	793	2	9.5	5.6	0.25
ii) X-rays									
Control	118	0	234	0	21342	0	..	..	..
5.0 krad	126	14	238	19	15263	91	11.1	8.0	0.60
7.5 ,,	123	23	240	26	14383	129	18.8	10.8	0.89
10.0 ,,	125	27	250	28	14202	118	21.6	11.2	0.83
12.5 ,,	125	24	249	29	12329	153	19.2	11.6	1.24
15.0 ,,	115	28	229	34	11802	153	24.3	14.8	1.30
17.5 ,,	125	31	249	37	10624	181	24.8	14.9	1.70
20.0 ,,	123	29	241	32	9652	147	23.6	13.3	1.52
22.5 ,,	123	32	242	38	9278	160	26.0	15.7	1.72
25.0 ,,	113	23	224	25	7940	172	20.4	11.2	2.16
30.0 ,,	104	25	200	30	7059	224	24.0	15.0	3.16
35.0 ,,	112	28	223	37	8137	271	25.0	16.6	3.33
40.0 ,,	73	17	135	20	4066	140	23.3	14.8	3.44
iii) Fast neutrons									
Control	120	0	235	0	16437	0	..	..	..
705 rad	120	22	238	24	11410	132	18.3	10.1	1.16
968 ,,	120	19	239	21	10020	87	15.8	8.8	0.87
1170 ,,	120	25	240	30	10364	107	20.8	12.5	1.03
1408 ,,	120	27	239	33	8882	245	22.5	13.8	2.76
1570 ,,	120	21	239	29	7250	150	17.5	12.1	2.07
1710 ,,	118	29	232	33	6647	174	24.6	14.2	2.62
1880 ,,	120	33	234	37	6262	158	27.5	15.8	2.52
2100 ,,	119	24	237	30	6287	85	20.2	12.7	1.35
iv) DES									
Control	90	0	180	0	13406	0	..	..	..
11.4 mM	98	6	195	7	13675	12	6.1	3.6	0.09
22.8 ,,	98	5	194	6	12888	15	5.1	3.1	0.11
34.2 ,,	102	4	201	4	12898	13	3.9	2.0	0.10
45.6 ,,	98	7	192	8	13545	18	7.1	4.2	0.13
57.0 ,,	97	13	191	17	12289	50	13.4	8.9	0.40

TABLE VII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
v) EMS									
Control	90	0	180	0	16564	0	..	..	..
19 mM	90	8	179	9	15888	13	8.9	5.0	0.08
38 ,,	90	10	178	12	15338	28	11.1	6.7	0.18
58 ,,	90	19	179	22	14440	82	21.1	12.3	0.57
77 ,,	89	16	178	16	15180	57	18.0	9.0	0.38
96 ,,	89	23	175	27	14692	191	25.8	15.4	1.30
115 ,,	90	18	179	20	15213	124	20.0	11.2	0.81
154 ,,	100	32	197	40	12349	249	32.0	20.3	2.02
192 ,,	100	36	197	45	12555	392	36.0	22.8	3.12
240 ,,	100	38	197	44	11911	296	38.0	22.3	2.48
288 ,,	100	48	197	64	12241	437	48.0	32.5	3.57
336 ,,	105	45	205	56	12803	329	42.9	27.3	2.57
384 ,,	110	45	219	58	12645	397	40.9	26.5	3.14
vi) MMS									
Control	90	0	178	0	16372	0	..	..	..
1.2 mM	90	0	175	0	14217	0	..	..	..
2.4 ,,	88	1	175	1	14747	2	1.1	0.6	0.01
3.6 ,,	90	1	177	1	15790	2	1.1	0.6	0.01
4.8 ,,	89	1	178	2	15788	8	1.1	1.1	0.05
5.9 ,,	90	7	180	7	15599	40	7.8	3.9	0.26
7.1 ,,	90	6	179	7	16466	77	6.7	3.9	0.47
8.3 ,,	100	9	199	13	14252	91	9.0	6.5	0.64
9.5 ,,	100	5	200	5	13927	41	5.0	2.5	0.29
11.8 ,,	100	8	200	8	13851	73	8.0	4.0	0.53
14.2 ,,	100	9	200	9	14065	48	9.0	4.5	0.34
17.7 ,,	100	9	200	9	13451	50	9.0	4.5	0.37
23.6 ,,	100	12	199	13	14402	74	12.0	6.5	0.51
29.5 ,,	100	13	198	17	14010	72	13.0	8.6	0.51
vii) NMU									
Control	90	0	180	0	12418	0	..	..	..
0.97 mM	90	16	178	19	10838	91	17.8	10.7	0.84
1.94 ,,	90	20	180	24	11921	136	22.2	13.3	1.14
2.91 ,,	89	21	177	26	11021	133	23.6	14.1	1.21
3.88 ,,	89	19	176	23	11214	103	21.4	13.1	0.92
4.85 ,,	90	18	176	19	11161	135	20.0	10.8	1.21
5.82 ,,	90	27	180	34	12052	189	30.0	18.9	1.57
7.76 ,,	57	24	112	33	7402	222	42.1	29.5	3.00
9.70 ,,	27	9	53	12	3549	100	33.3	22.6	2.82
viii) MNNG									
Control	90	0	180	0	13472	0	..	..	..
0.68 mM	90	2	180	3	12590	8	2.2	1.7	0.06
1.36 ,,	90	4	180	5	12271	13	4.4	2.8	0.10
2.72 ,,	90	3	179	3	12812	5	3.3	1.7	0.04
4.08 ,,	90	5	179	5	12971	11	5.6	2.8	0.08
5.44 ,,	90	8	177	6	12958	52	5.6	3.4	0.40
6.80 ,,	90	8	177	9	12213	36	8.9	5.1	0.29
8.16 ,,	90	2	180	2	12858	6	2.2	1.1	0.05
10.20 ,,	90	1	176	1	12862	1	1.1	0.6	0.01

TABLE VIII

Relative percentages of different types (spectrum) of chlorophyll mutants in the M_2 generation

Mutagen and dose	Total No. of mutants	Relative percentages of chlorophyll mutants							
		A	X	V	C	AV	S	T	Others*
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i) Gamma rays									
10 krad	77	61	3	9	9	..	18	..	..
20 "	64	44	..	25	17	..	14	..	..
30 "	32	22	..	34	44	..	..	..	..
40 "	135	38	7	7	34	..	14	..	..
50 "	2	50	..	50	..	..	..	..	..
Total	310	43	4	14	25	..	14	..	..
ii) X-rays									
5.0 krad	91	44	..	40	1	6	1	..	8
7.5 "	129	46	7	25	21	1	..	..	..
10.0 "	118	38	4	47	3	5	..	..	3
12.5 "	153	31	4	22	8	10	1	..	24
15.0 "	153	31	1	40	10	13	5	..	..
17.5 "	181	49	1	24	9	5	7	1	4
20.0 "	147	48	9	27	10	5	..	..	1
22.5 "	160	41	3	23	20	10	1	..	2
25.0 "	172	33	8	35	20	1	1	..	2
30.0 "	224	27	13	43	9	7	1	..	..
35.0 "	271	45	1	19	20	13	1	..	1
40.0 "	140	35	1	36	4	7	13	..	4
Total	1939	39	4	31	12	8	2	..	4
iii) Fast neutrons									
705 rad	132	27	15	21	20	15	2	..	..
968 "	87	24	3	22	1	10	4	16	20
1170 "	107	46	2	36	7	3	6	..	..
1408 "	245	44	2	37	10	3	4	..	..
1570 "	150	61	5	16	17	1	..	..	..
1710 "	174	45	..	29	11	2	4	..	9
1880 "	158	39	6	28	15	1	11	..	..
2100 "	85	59	6	12	13	8	1	1	..
Total	1138	43	5	27	12	5	4	1	3

* M, VA, XA and EL

TABLE VIII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
iv) DES									
11.4 mM	12	25	17	41	..	..	17	..	..
22.8 "	15	20	40	13	..	..	27	..	..
34.2 "	13	46	..	54	..	..	..	..	..
45.6 "	18	..	17	61	22	..	..	..	..
57.0 "	50	28	4	58	2	..	8	..	..
Total	108	24	12	50	5	..	9	..	..
v) EMS									
19 mM	13	..	8	92	..	..	..	..	..
38 "	28	..	7	79	14	..	..	..	..
58 "	82	7	28	50	14	..	1	..	..
77 "	57	..	23	61	7	7	2	..	..
96 "	191	26	35	23	16	..	..	..	..
115 "	124	49	3	28	..	20	..	..	..
154 "	249	31	5	36	14	3	10	..	3
192 "	392	8	6	43	8	11	..	7	17
240 "	296	24	10	29	19	15	3	..	..
288 "	437	36	2	42	10	5	4	1	..
336 "	329	11	10	45	19	6	..	1	8
384 "	397	33	8	38	8	5	..	..	8
Total	2595	24	10	39	12	7	2	1	5
vi) MMS									
1.2 mM	..	..	..	..	..	..	..	..	..
2.4 "	2	..	..	..	..	..	100	..	..
3.6 "	2	100	..	..	..	..	..	..	..
4.8 "	8	100	..	..	..	..	..	..	..
5.9 "	40	85	3	12	..	..	..	..	..
7.1 "	77	18	..	47	35	..	..	..	..
8.3 "	91	48	1	..	9	13	1	..	28
9.5 "	41	7	..	7	30	..	..	..	56
11.8 "	73	5	3	5	84	3	..	..	..
14.2 "	48	33	..	2	8	57	..	..	..
17.7 "	50	46	2	46	2	4	..	..	..
23.6 "	74	48	..	30	20	2	..	..	..
29.5 "	72	12	11	29	24	18	6	..	..
Total	578	33	2	21	25	10	1	..	8
vii) MMU									
0.97 mM	91	10	..	65	..	20	..	5	..
1.94 "	136	36	2	43	5	10	..	..	4
2.91 "	135	24	5	22	17	11	9	..	12
3.88 "	103	18	3	25	27	2	..	..	25
4.85 "	135	20	17	30	7	18	..	..	8
5.82 "	189	24	..	29	38	2	7	..	..
7.76 "	222	8	26	48	1	11	4	1	1
9.70 "	100	2	..	65	27	1	5	..	..
Total	1109	18	8	40	15	9	3	1	6

TABLE VIII (CONTD.)

(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
viii)MRNG										
0.68	MM	8	..	..	100	..	..	..	..	..
1.36	..	13	23	..	46	31	..	..	..	..
2.72	..	5	20	..	60	..	..	20	..	..
4.08	..	11	..	..	46	27	..	..	..	27
5.44	..	52	..	2	86	10	..	..	..	2
6.80	..	36	..	..	53	36	11	..	..	..
8.16	..	6	..	..	100	..	..	..	..	..
10.20	..	1	100	..	..	..	..	..	..	..
Total		132	4	1	69	19	3	1	..	3

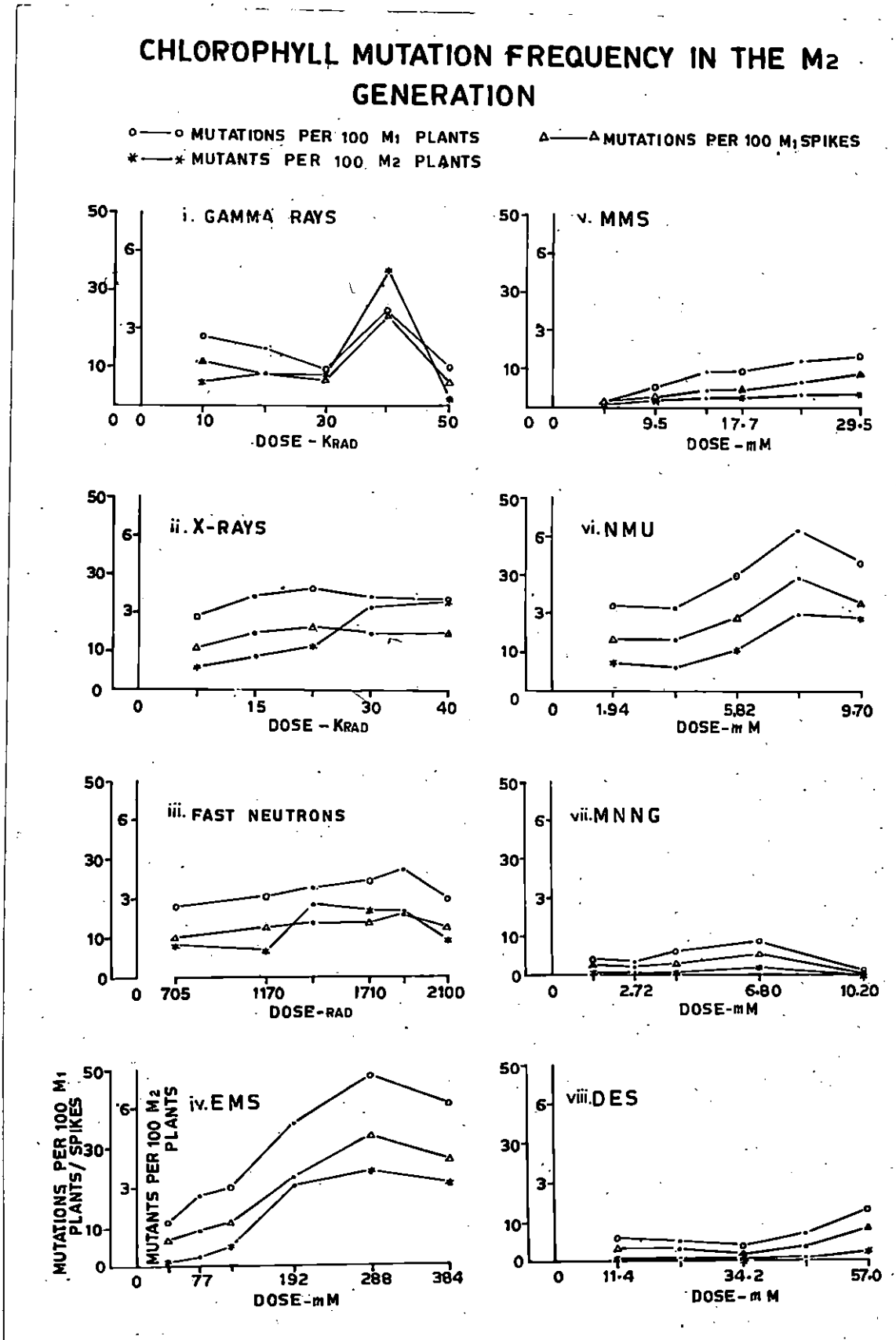

CHLOROPHYLL MUTATION FREQUENCY IN THE M2 GENERATION
o—o MUTATIONS PER 100 M1 PLANTS
— MUTANTS PER 100 M2 PLANTS
Δ—Δ MUTATIONS PER 100 M1 SPIKES
i. GAMMA RAYS
DOSE - KRAD
ii. X-RAYS
DOSE - KRAD
iii. FAST NEUTRONS
DOSE-RAD
iv. EMS
MUTATIONS PER 100 M1 PLANTS/ SPIKES
MUTANTS PER 100 M2 PLANTS
DOSE-mM
v. MMS
DOSE - mM
vi. NMU
DOSE-m M
vii. MNNG
DOSE-mM
viii. DES
DOSE-m M

Figure 7

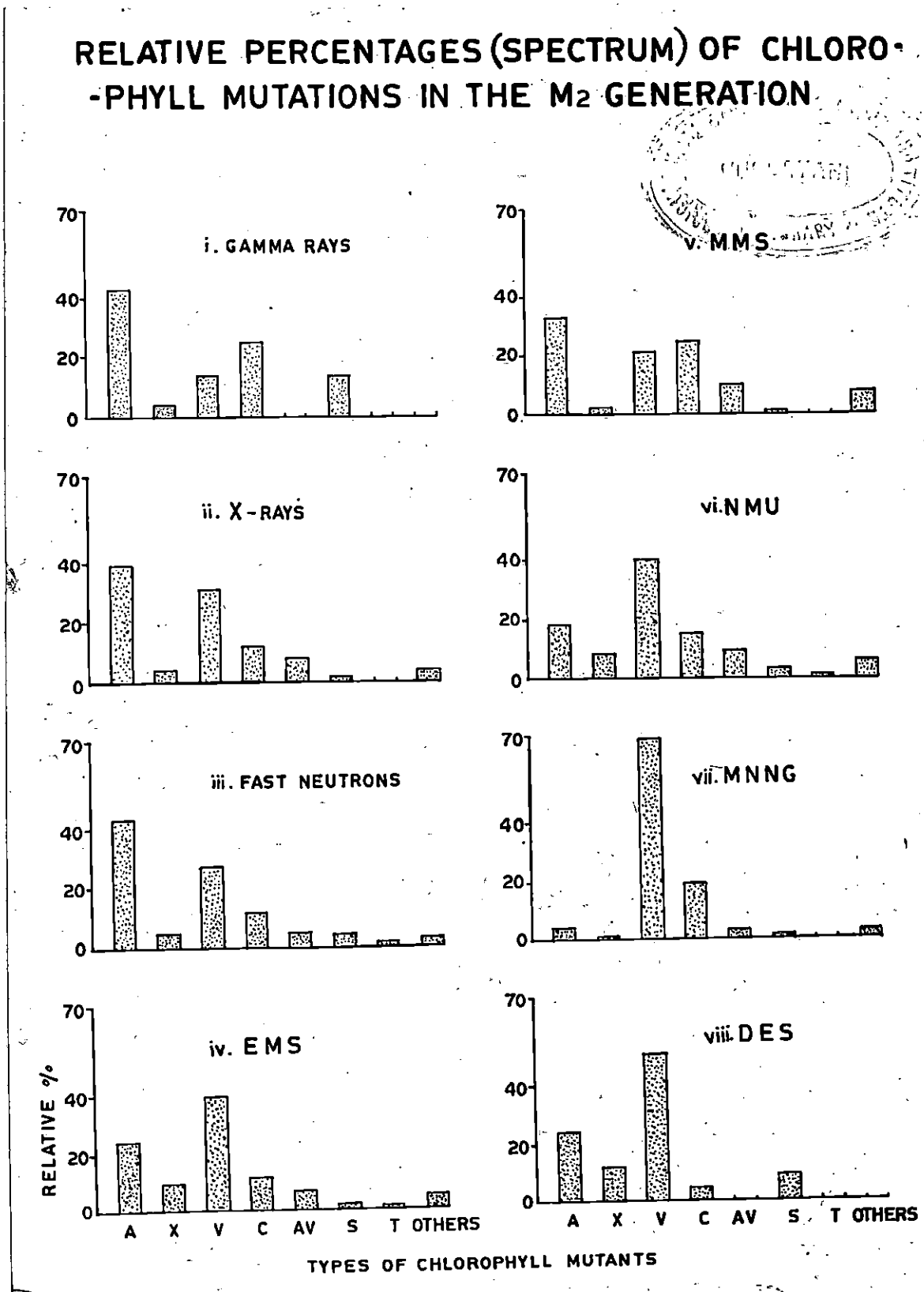

RELATIVE PERCENTAGES (SPECTRUM) OF CHLORO-PHYLL MUTATIONS IN THE M2 GENERATION
i. GAMMA RAYS
ii. X-RAYS
iii. FAST NEUTRONS
iv. EMS
v. MMS
vi. NMU
vii. MNNG
viii. DES
RELATIVE %
70
40
20
0
A X V C AV S T OTHERS
TYPES OF CHLOROPHYLL MUTANTS

Figure 8

radiation treatments, whereas viridis was predominant in treatments with chemical mutagens. A reduction in albina was generally followed by an increase in viridis. Differences in spectra were not conspicuous between doses of the same mutagen. Albinas and xanthas were always lethal. Certain of the viridis, chlorina and alboviridis mutants were lethal and the others viable. Most of the types under striata, tigrina, maculata and brown lamina were viable. In each of the above classes of chlorophyll mutants several different types were identified based on the intensity of colour, differences in pattern of colour distribution, stature of plants and the intensity of the lethal effect. These different types might be governed by different genes.

Certain ear-progenies were found to segregate for more than one type of mutation. The frequencies and relative percentages of ear-progenies segregating for multiple mutations were estimated dose wise and presented in Table IX. The mean number of mutations per mutated ear increased with increasing doses of the mutagens. This was due to an increase in the frequency of ear-progenies with two and three types of mutations. The rate of increase in the frequency of multiple mutations, however, was not proportional to the dose. The highest frequencies were recorded in treatments with EMS.

The relative percentages of M_1 ear-progenies with different frequencies of recessive mutants and the percentage of mutants in mutated ears are presented in Table X. With increasing doses of radiations as well as chemical mutagens

TABLE IX

Frequency and percentage of M_1 ear progenies segregating for single and multiple chlorophyll mutations

Mutagen and dose	No. of ear progenies segregating	Chlorophyll mutations		Ear progenies segregating for mutations of:					
		Total No.	Mean No. per ear	One type	Two types	Three types	One type	Two types	Three types
				Frequency			Relative %		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i) Gamma rays									
10 krad	22	23	1.05	21	1	..	95	5	..
20 ,,	14	16	1.14	12	2	..	86	14	..
30 ,,	9	11	1.22	8	..	1	89	..	11
40 ,,	27	31	1.15	23	4	..	85	15	..
50 ,,	2	2	1.00	2	..	..	100	..	..
Total	74	83	1.12	66	7	1	89	10	1
ii) X-rays									
5.0 krad	19	21	1.11	17	2	..	90	10	..
7.5 ,,	26	28	1.08	24	2	..	92	8	..
10.0 ,,	28	30	1.07	26	2	..	93	7	..
12.5 ,,	29	34	1.17	25	3	1	87	10	3
15.0 ,,	34	36	1.06	32	2	..	94	6	..
17.5 ,,	37	39	1.05	35	2	..	94	6	..
20.0 ,,	32	33	1.03	31	1	..	97	3	..
22.5 ,,	38	39	1.03	37	1	..	97	3	..
25.0 ,,	25	25	1.00	25	..	..	100	..	..
30.0 ,,	30	31	1.03	29	1	..	97	3	..
35.0 ,,	37	39	1.06	35	2	..	94	6	..
40.0 ,,	20	22	1.10	18	2	..	90	10	..
Total	355	377	1.06	334	20	1	94	6	..
iii) Fast neutrons									
705 rad	24	24	1.00	24	..	..	100	..	..
968 ,,	21	21	1.00	21	..	..	100	..	..
1170 ,,	30	32	1.07	28	2	..	94	6	..
1408 ,,	33	35	1.06	31	2	..	94	6	..
1570 ,,	29	33	1.14	25	4	..	86	14	..
1710 ,,	33	35	1.06	31	2	..	94	6	..
1880 ,,	37	39	1.05	35	2	..	94	6	..
2100 ,,	30	32	1.07	28	2	..	94	6	..
Total	237	251	1.06	223	14	..	94	6	..

TABLE IX (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
iv) EMS									
19 mM	9	9	1.00	9	..	..	100	..	..
38 ,,	12	12	1.00	12	..	..	100	..	..
58 ,,	22	24	1.09	20	2	..	91	9	..
77 ,,	16	18	1.12	14	2	..	88	12	..
96 ,,	27	28	1.04	26	1	..	96	4	..
115 ,,	20	21	1.05	19	1	..	95	5	..
154 ,,	40	52	1.30	30	8	2	75	20	5
192 ,,	45	55	1.22	37	6	2	82	13	5
240 ,,	44	60	1.36	31	10	3	70	23	7
288 ,,	64	80	1.25	49	14	1	76	22	2
336 ,,	56	71	1.27	42	13	1	75	23	2
384 ,,	58	71	1.22	47	9	2	81	16	3
Total	413	501	1.21	336	66	11	81	16	3
v) NMS									
1.2 mM	..	..	..	..	..	..	..	..	..
2.4 ,,	1	1	1.00	1	..	..	100	..	..
3.6 ,,	1	1	1.00	1	..	..	100	..	..
4.8 ,,	2	2	1.00	2	..	..	100	..	..
5.9 ,,	7	7	1.00	7	..	..	100	..	..
7.1 ,,	7	7	1.00	7	..	..	100	..	..
8.3 ,,	21	22	1.05	20	1	..	95	5	..
9.5 ,,	5	6	1.20	4	1	..	80	20	..
11.8 ,,	8	9	1.13	7	1	..	88	12	..
14.2 ,,	9	9	1.00	9	..	..	100	..	..
17.7 ,,	9	9	1.00	9	..	..	100	..	..
23.6 ,,	13	14	1.08	12	1	..	92	8	..
29.5 ,,	17	20	1.18	14	3	..	82	18	..
Total	100	107	1.07	93	7	..	93	7	..
vi) NMU									
0.97 mM	19	19	1.00	19	..	..	100	..	..
1.94 ,,	24	27	1.12	21	3	..	88	12	..
2.91 ,,	26	29	1.11	23	3	..	88	12	..
3.88 ,,	23	24	1.04	22	1	..	96	4	..
4.85 ,,	19	22	1.16	16	3	..	84	16	..
5.82 ,,	34	35	1.03	33	1	..	97	3	..
7.76 ,,	33	37	1.12	29	4	..	88	12	..
9.70 ,,	12	13	1.08	11	1	..	92	8	..
Total	190	206	1.08	174	16	..	92	8	..

TABLE X

Segregation percentages of chlorophyll mutations in the M_2 generation

Mutagen and dose	Total No. of mutations	Relative percentages of M_1 ear progenies with recessive mutant percentages of:							% of mutants in mutated ears
		<5	5-10	10-15	15-20	20-25	25-30	>30	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
i) Gamma rays									
10 krad	23	47	22	9	9	9	..	4	7.87
20 ,,	16	25	13	12	12	19	19	..	14.00
30 ,,	11	9	27	37	..	9	..	18	18.50
40 ,,	31	10	13	16	16	16	3	26	20.33
50 ,,	2	..	..	..	50	50	..	..	22.22
ii) X-rays									
5.0 krad	21	43	28	19	5	..	5	..	7.36
7.5 ,,	28	42	35	11	4	4	4	..	7.72
10.0 ,,	30	37	37	16	..	10	..	..	7.47
12.5 ,,	34	41	23	21	15	..	..	..	9.11
15.0 ,,	36	16	28	25	6	11	6	8	12.44
17.5 ,,	39	36	20	18	13	7	3	3	9.69
20.0 ,,	33	28	24	21	18	3	..	6	9.65
22.5 ,,	39	15	18	30	13	8	13	3	12.23
25.0 ,,	25	16	24	16	12	12	16	4	14.36
30.0 ,,	31	13	22	13	10	19	10	13	16.87
35.0 ,,	39	10	13	28	15	18	8	8	19.65
40.0 ,,	22	9	23	32	14	..	18	4	18.59
iii) Fast neutrons									
705 rad	24	29	25	13	25	4	4	..	10.96
968 ,,	21	33	24	14	19	5	5	..	10.88
1170 ,,	32	47	25	9	6	13	..	..	8.46
1408 ,,	35	23	17	20	14	14	9	3	16.59
1570 ,,	33	28	24	15	15	12	..	6	14.18
1710 ,,	35	9	17	31	17	14	6	6	15.20
1880 ,,	39	8	28	13	33	8	8	2	14.79
2100 ,,	32	25	35	22	3	9	3	3	9.30

2100

TABLE X (CONTD.)

(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
iv) EMS										
19	mM	9	100	..	..	..	..	..	..	1.59
38	,,	12	75	8	17	..	..	..	..	3.50
58	,,	24	66	21	..	13	..	..	..	4.95
77	,,	18	61	39	..	..	..	..	..	4.21
96	,,	28	53	4	7	11	18	7	..	9.44
115	,,	21	62	14	14	10	..	..	..	6.54
154	,,	52	38	19	17	10	10	2	4	11.06
192	,,	55	29	13	16	16	13	7	6	15.67
240	,,	60	38	25	15	5	5	7	5	12.87
288	,,	80	21	30	15	10	13	5	6	14.58
336	,,	71	39	22	20	9	6	1	3	10.82
384	,,	71	21	17	23	15	10	7	7	14.33
v) HMU										
0.97	mM	19	42	42	..	5	11	..	..	7.37
1.94	,,	27	40	19	18	15	4	4	4	8.95
2.91	,,	29	41	31	10	14	4	..	..	8.03
3.88	,,	24	50	29	9	4	4	4	..	6.67
4.85	,,	22	36	18	13	18	5	5	5	11.10
5.82	,,	35	46	23	11	11	9	..	..	7.85
7.76	,,	37	49	14	19	5	5	3	5	9.94
9.70	,,	13	31	31	8	15	..	15	..	13.14

the frequency of ear-progenies with higher segregation ratios had increased. The number of ear-progenies with frequencies of mutants deviating from 25 per cent were more in treatments with chemical mutagens and lower doses of radiations. The mean segregation ratios also increased with increasing doses of the mutagens. In treatments with gamma rays and X-rays the mean segregation ratios approached the theoretical maximum of 25 per cent at the higher doses, whereas with fast neutrons, EMS and NMU the ratios increased with doses up to a critical level beyond which there was a saturation effect. The segregation ratios provided information on the chimeric nature of M_1 ears.

b. Lethal seedling and nonviable mutations

Several types of lethal seedling mutants with strongly inhibited seedling growth were recorded (Figure 9). They appeared in the early seedling stage but could not be maintained for over a long period in spite of special attention and care. Lethality in these mutants might be due to strong genetic inhibitions affecting growth and differentiation. They were induced more frequently with fast neutrons than with sparsely ionising radiations and chemical mutagens.

The nonviable mutants were capable of surviving beyond the seedling age and some could survive up to the stage of maturity. But they failed to produce normal panicles. Several such types were recorded and maintained with special care (Figures 10 and 11). Nonviable mutants were more frequently induced with radiations than with chemical mutagens.

Figure 9. Lethal seedling mutants (20 days old)

Induced by gamma rays

1. Minute - short, broad, dark green leaves

Induced by fast neutrons

3. Stunted with tubular leaves

5. Stunted with narrow leaves

6. Highly dwarf with thick coarse leaves

7. Highly stunted

8. Highly dwarf, weak and narrow leaved

Induced by EMS

9. Dwarf with tubular leaves

Induced by NMU

2. Stunted with proliferated leaves

4. Highly stunted

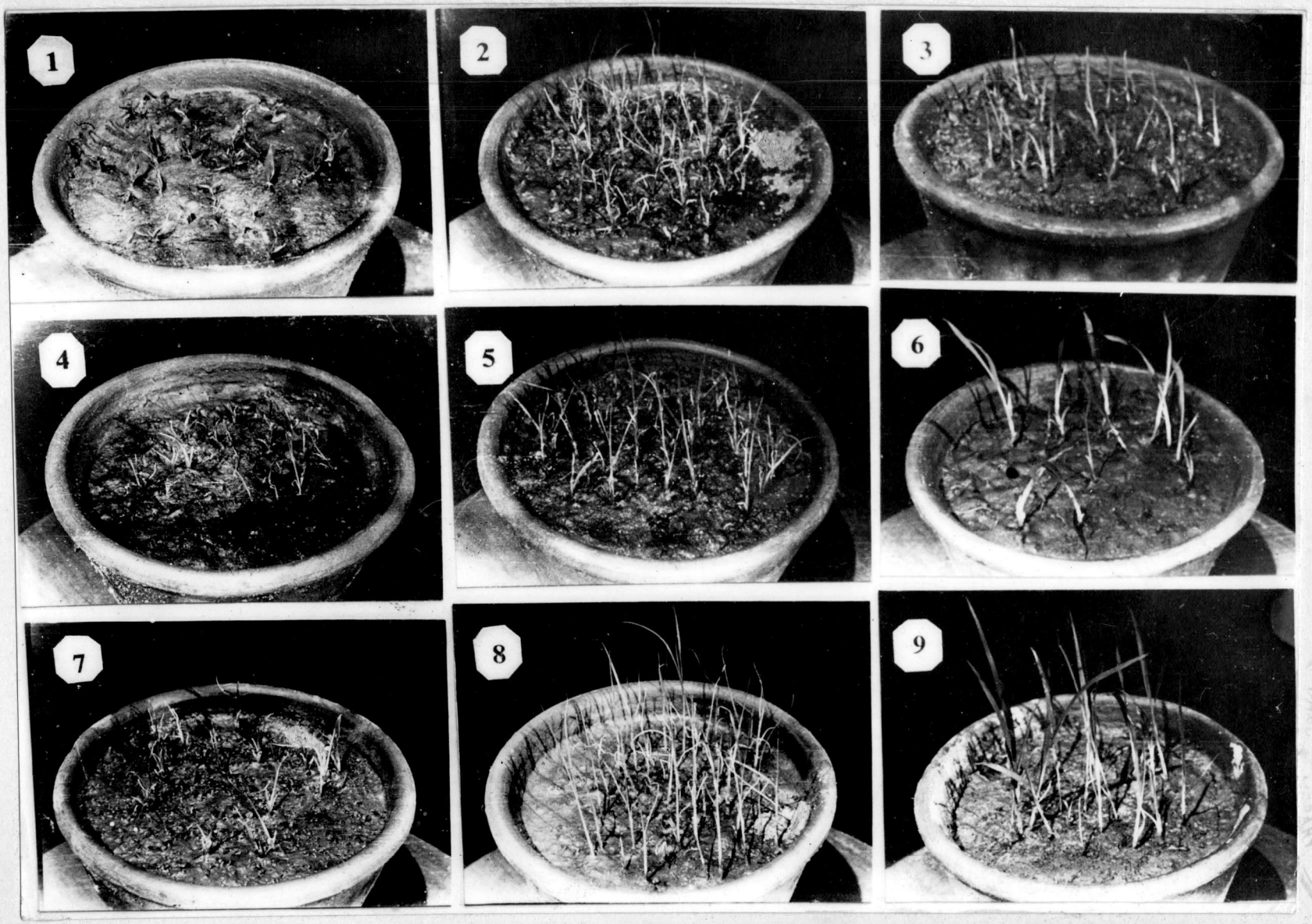


Figure 9

Figure 10. Nonviable mutants - I (Seedlings - 30 days old)

C. Control.

Induced by gamma rays

1. Dwarf with narrow leaves.

Induced by fast neutrons

5. Dwarf with twisted tubular leaves.

7. Tall, weak with elongated culm and leaves.

8. Dwarf.

Induced by EMS

3. Dwarf with ageotropic habit.

4. Dwarf with short, broad leaves.

6. Dwarf with ageotropic habit.

9. Dwarf with rolled leaves.

10. Semidwarf with ageotropic habit.

Induced by NMU

2. Dwarf.

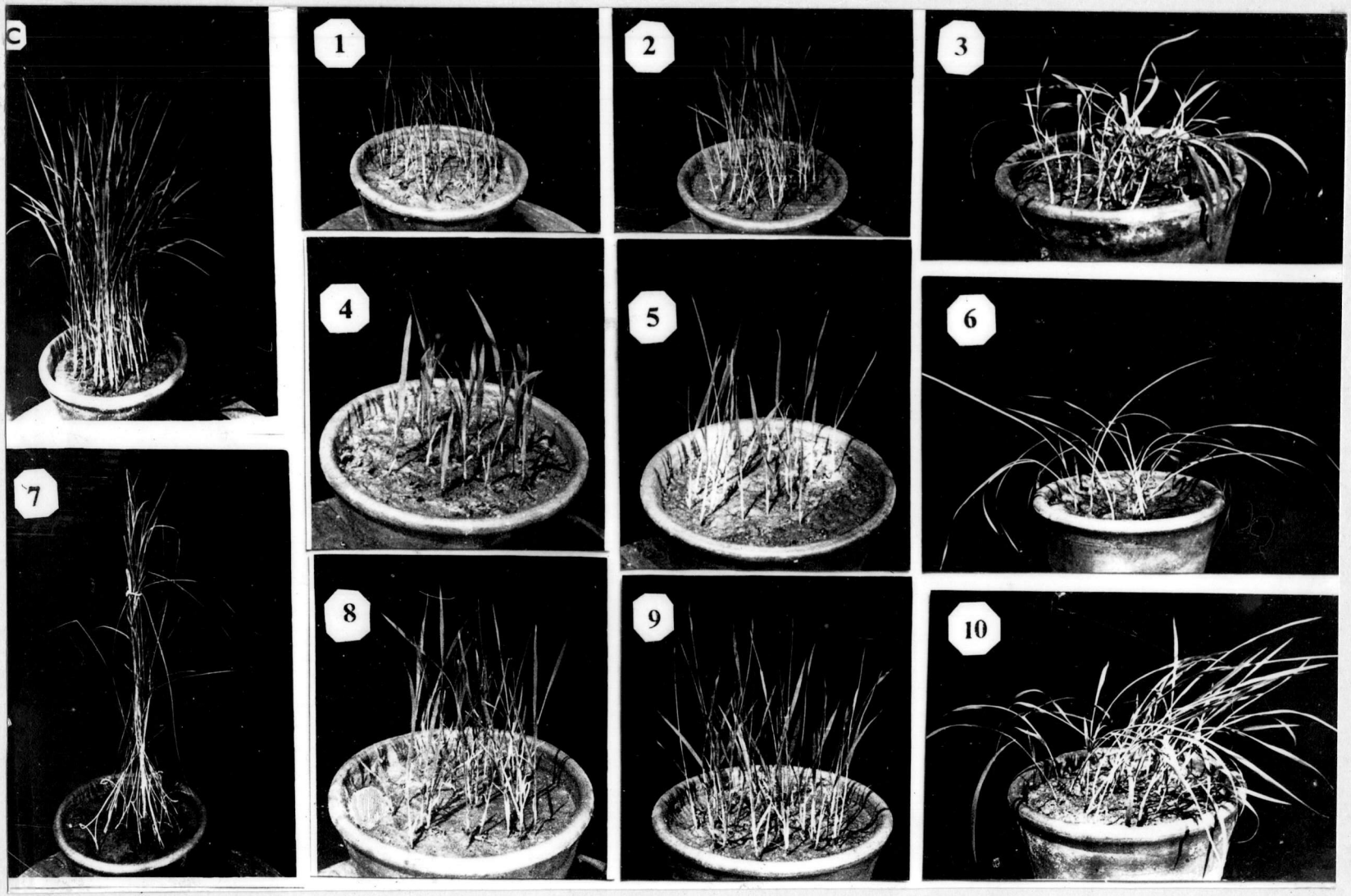


Figure 10

Figure 11. Nonviable mutants - II (Plants 100 days old).

C. Control

Induced by gamma rays

1. Dwarf, high tillering with flat culm and two ranked leaves - nonflowering.
8. Dwarf with thin leaves.
9. Dwarf.
10. Highly dwarf - nonflowering.
11. Dwarf with open tillers and broad leaves.
14. Dwarf.
15. Dwarf with short leaf sheath and long lamina.
17. Highly dwarf - nonflowering.

Induced by fast neutrons

2. Tall, low tillering with flat culm and two ranked leaves - nonflowering.
4. Dwarf with thick culm and short broad coarse leaves. Short compact panicle.
12. Dwarf.
13. Dwarf with short broad leaves.
16. Dwarf with open tillers.

Induced by EMS

3. Stunted with short broad dark green leaves - nonflowering.

Induced by NMU

5. Dwarf.
6. Dwarf.
7. Dwarf with malformed leaves.

Figure 11

c. Viable mutations

The study of viable mutations was confined to four mutagens viz., gamma rays, fast neutrons, EMS and HMU since they were found to be more effective than the others in inducing chlorophyll mutations. All mutations affecting the morphology of the different plant parts except chlorophyll deficient types were classed as viable mutations. These were detectable in individual plants in the M_2 generation by visual observation and were capable of successfully completing the generation. The induced changes affected one or more characters of a plant at the same time.

i) Frequency

The viable mutation frequency was estimated as mutations per 100 M_1 spikes. The frequencies were found to increase with increasing doses of radiations as well as chemical mutagens as could be seen from Table XI. In treatments with radiations the frequencies decreased at the highest dose, whereas with chemical mutagens the frequencies progressively increased upto the highest dose employed. The highest frequency of viable mutations was induced by gamma rays followed by EMS, HMU and fast neutrons in decreasing order.

ii) Relative frequency of different types (spectrum)

A wide spectrum of mutations affecting various morphological characters such as height, duration, leaf, panicle, grain, and panicle and spikelet development could be isolated. The relative percentages of the different types of mutations were estimated from the total number of mutations induced

TABLE XI

Frequency of viable and total mutations in the M_2 generation

Mutagen and dose	Viable mutations			Total mutations		
	No. of M_1 ear progenies		Mutations per 100 M_1 spikes (ears)	No. of M_1 ear progenies		Mutations per 100 M_1 spikes (ears)
	Scored	Segregating		Scored	Segregating	
i) Gamma rays						
Control	120	0	..	120	0	..
✓ 10 krad	113	13	11.5	113	24	21.2
✓ 20 ,,	102	30	29.4	102	36	35.3
✓ 30 ,,	91	33	36.3	91	40	44.0
✓ 40 ,,	65	32	49.2	65	41	63.1
✓ 50 ,,	36	17	47.2	36	18	50.0
ii) Fast neutrons						
Control	120	0	..	120	0	..
✓ 705 rad	119	8	6.7	119	20	16.8
968 ,,	119	15	12.6	119	24	20.2
✓ 1170 ,,	120	16	13.3	120	29	24.2
✓ 1408 ,,	119	16	13.3	119	33	27.7
1570 ,,	119	22	18.5	119	31	26.1
✓ 1710 ,,	115	19	16.5	115	37	32.2
1880 ,,	116	29	25.0	116	42	36.2
✓ 2100 ,,	114	15	13.2	114	27	23.7
iii) EMS						
Control	120	0	..	120	0	..
19 mM	±118	±2	±1.7	118	9	7.6
38 ,,	118	4	3.4	118	13	11.0
✓ 58 ,,	±120	±10	±8.3	120	22	18.3
77 ,,	118	8	6.8	118	21	17.8
96 ,,	±117	±11	±9.4	117	27	23.1
✓ 115 ,,	119	17	14.3	119	31	26.1
154 ,,	118	24	20.3	118	39	33.1
✓ 192 ,,	120	30	25.0	120	54	45.0
✓ 240 ,,	118	37	31.3	118	51	43.2
✓ 288 ,,	118	41	34.7	118	61	51.7
iv) NMU						
Control	120	0	..	120	0	..
0.97 mM	118	13	11.0	118	23	19.5
✓ 1.94 ,,	120	18	15.0	120	32	26.7
2.91 ,,	118	13	11.0	118	27	22.9
✓ 3.88 ,,	117	18	15.4	117	31	26.5
4.85 ,,	119	15	12.6	119	28	23.5
✓ 5.82 ,,	120	29	24.2	120	52	43.3
✓ 7.76 ,,	113	19	16.8	113	48	42.5
✓ 9.70 ,,	53	17	32.1	53	26	49.1

by each mutagen over all the doses combined. The data are summarised in Table XII.

The types of mutations induced did not differ significantly with the mutagens. But the relative frequencies with which they were induced differed. There was a predominance of macro-mutants and mutants effecting culm length among the induced types. Erectoides and other macro-mutants were very frequently induced with fast neutrons and NMU. Mutants affecting culm length were common among the types induced by EMS and mutants with altered duration were larger in number following treatment with gamma rays. Mutants for grain type were more commonly induced than others with gamma rays and NMU, whereas abnormal panicle and spikelet mutants were of frequent occurrence after treatment with fast neutrons. The proportion of erectoides among the macro-mutants was large in treatment with fast neutrons. Dwarfs and semi-dwarfs among the mutants affecting culm length and long duration types among the mutants with altered maturity period were predominant. Mutants with enhanced effects such as tall stature and long duration were induced very frequently by EMS and gamma rays respectively.

iii) Types of mutants

A total number of 421 viable mutants was isolated in the present study. They have been classified as macro-mutants, visible mutants and systematic mutants.

1) Macro-mutants

Mutants in which the change though inherited as a single unit of recombination, manifested on several characters

TABLE XII

Relative frequencies and percentages of different types (spectrum) of viable mutations in the M_2 generation

Mutants	Frequency				Relative percentages			
	Gamma rays	Fast neutrons	EMS	NMU	Gamma rays	Fast neutrons	EMS	NMU
i) Macro-mutants								
Erectoides	2	19	12	11	1.3	11.6	5.2	7.5
Others	47	63	70	54	31.8	38.4	30.4	37.0
ii) Height mutants								
Dwarf	8	12	15	12	5.4	7.3	6.5	8.2
Semi-dwarf	21	23	45	15	14.2	14.0	19.5	10.3
Tall	..	1	16	..	..	0.6	7.0	..
iii) Duration mutants								
Early	3	1	6	2	2.0	0.6	2.6	1.4
Late	12	3	7	6	8.1	1.8	3.0	4.1
Very late	1	..	2	..	0.7	..	0.9	..
iv) Leaf type mutants								
Narrow leaves	3	3	5	2	2.0	1.8	2.2	1.4
v) Ear mutants								
Compact	1	1	..	..	0.7	0.6	..	..
Long	5	2	3	1	3.4	1.2	1.3	0.7
vi) Grain type mutants								
Small	1	..	3	9	0.7	..	1.3	6.2
Medium	3	6	3	4	2.0	3.7	1.3	2.7
Bold	13	3	8	3	8.7	1.8	3.5	2.1
Fine	12	5	11	5	8.1	3.0	4.8	3.4
Large	1	3	5	6	0.7	1.8	2.2	4.1
vii) Grain colour mutants	6	2	6	2	4.0	1.2	2.6	1.4
viii) Abnormal panicle and spikelet mutants	7	16	8	10	4.7	9.8	3.5	6.8
ix) Other mutants	2	1	5	4	1.3	0.6	2.2	2.7
Total	148	164	230	146	..	..	..	..

simultaneously were grouped as macro-mutants. The most important among them was the erectoides mutants. They were either dwarf (Figure 12), semi-dwarf (Figure 13) or normal in stature with stiff culm, compact tillers, dark, broad, coarse, erect leaves and exerted compact ears with small or medium-sized bold grains (Figure 14). Twenty erectoides mutants differing in morphological characters were isolated.

A total of 216 macro-mutants belonging to several types other than erectoides were also recorded. Each mutant represented a combination of several altered characters with (Figures 15 to 18) or without (Figure 19) height reduction. In many of them the ears and grains were variously transformed. The ears varied from short compact ones to long open types (Figures 20 to 23). The grains also varied from very small to very large in size and from bold to long fine in type (Figure 24). Some mutants had awns of various lengths with or without grain size alterations (Figures 25 and 26).

2) Visible mutants

Each of these mutants represented alterations in a single plant character. Types with altered plant height (52 Nos.), duration (28 Nos.), leaf type (13 Nos.), ear type (12 Nos.), grain size (21 Nos.) and grain colour (9 Nos.) were recovered. Mutants for plant height ranged from dwarf to very tall ones. The great majority of them were either dwarf or semi-dwarf. Tall mutants (Figures 27 and 28) were relatively rare. Mutants with altered duration were either early (Figure 29), late or very late

Figure 12. Dwarf *erectoides* mutants

1. Control.
2. Thick culm, compact tillers, dark green short broad coarse leaves, exerted stiff compact ears with very small bold grains (Fig.14-6). - EMS.
3. Almost similar to No.2 with less green leaves and highly compact short ears. (Fig.14-2). - Gamma rays.
4. Highly dwarf, semisterile. Otherwise similar to No.2 - EMS.

Figure 13. Semi-dwarf *erectoides* mutants

1. Control
2. Compact tillers, compact ears with medium bold grains (Fig.14-7) - NMU.
3. Almost similar to No.2 with more compact tillers and short ears (Fig.14-8) - EMS.
4. Open tillers with compact ears and medium bold grains (Fig.14-9) - NMU.

Figure 14. Ears of *erectoides* mutants

C. Control

Nos. 1, 2 and 3 - Gamma rays

No. 4 - Fast neutrons

Nos. 5, 6 & 8 - EMS.

Nos. 7, 9 & 10 - NMU.

Figure 12

Figure 13

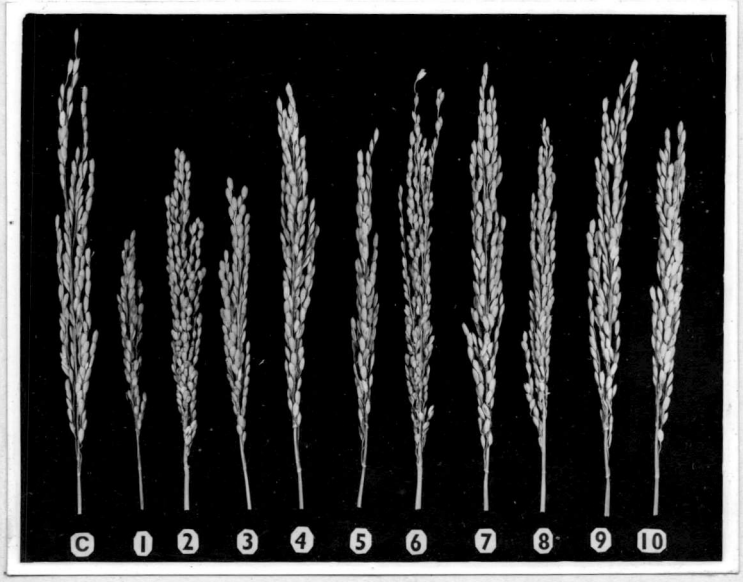


Figure 14

Figure 15. Dwarf macro-mutants.

C. Control.

Nos. 4, 5, 6, 7 and 16 - Gamma rays.

Nos. 10, 11 and 13 - Fast neutrons.

Nos. 1, 2, 3 and 12 - EMS.

Nos. 8, 9, 14, 15, 17 and 18 - NMU.

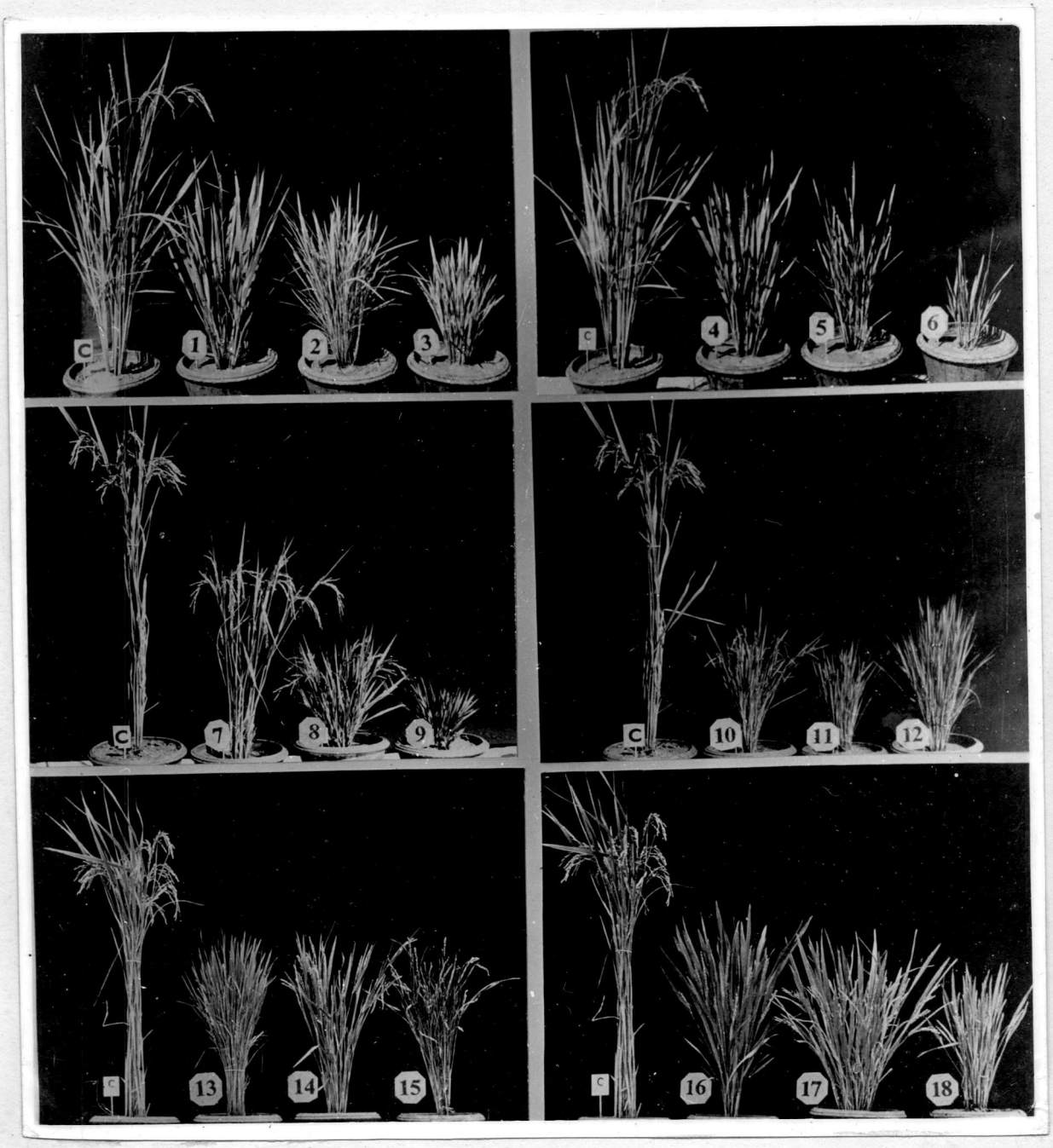


Figure 15

Figure 16. Semi-dwarf macro-mutants - I.

C. Control.

Nos. 1, 9, 14 and 17 - Gamma rays.

Nos. 2, 3, 4, 5, 6 and 8 - Fast neutrons.

Nos. 7, 13, 15 and 16 - EMS.

Nos. 10, 11, 12 and 18 - NMU.

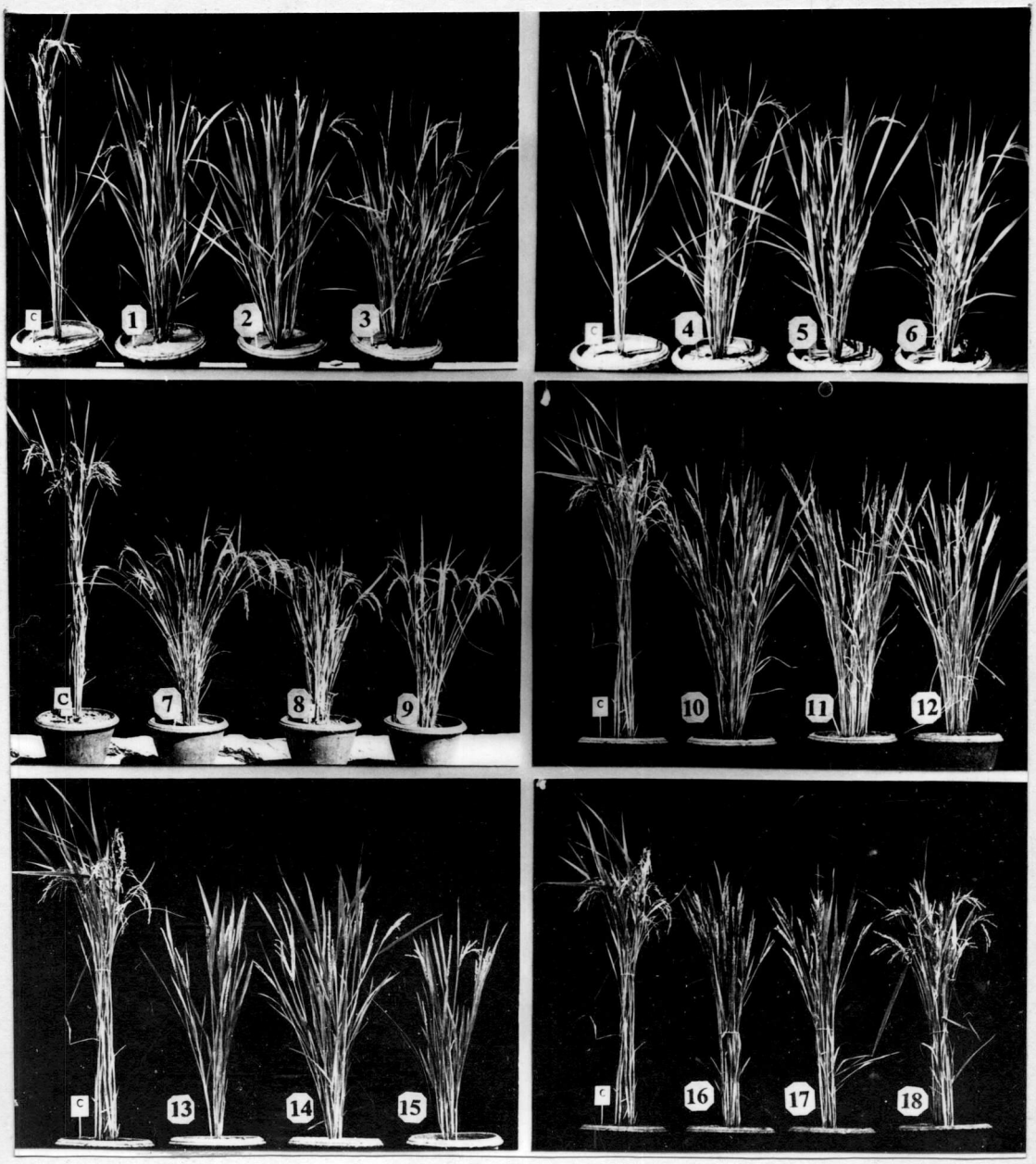


Figure 16.

Figure 17. Semi-dwarf macro-mutants - II.

1. Control.
2. Induced by fast neutrons.
3.and 4. Induced by EMS.

Figure 18. Semi-dwarf macro-mutants - III.

1. Control.
2 and 3. Induced by NMU.
4. Induced by fast neutrons.

Figure 19. Macro-mutants with normal height.

1. Control.
2. Induced by EMS.
3 and 4. Induced by fast neutrons.

Figure 17

Figure 18

Figure 19

Figure 20. Ears of macro-mutants - I.

C. Control.

Nos. 1 to 9. Induced by gamma rays.

Figure 21. Ears of macro-mutants - II.

C. Control.

Nos. 1 to 10. Induced by fast neutrons.

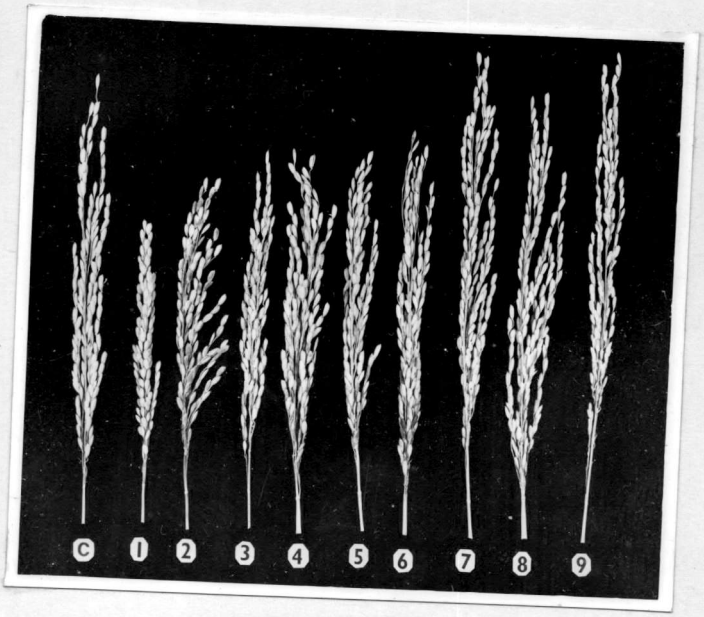


Figure 20

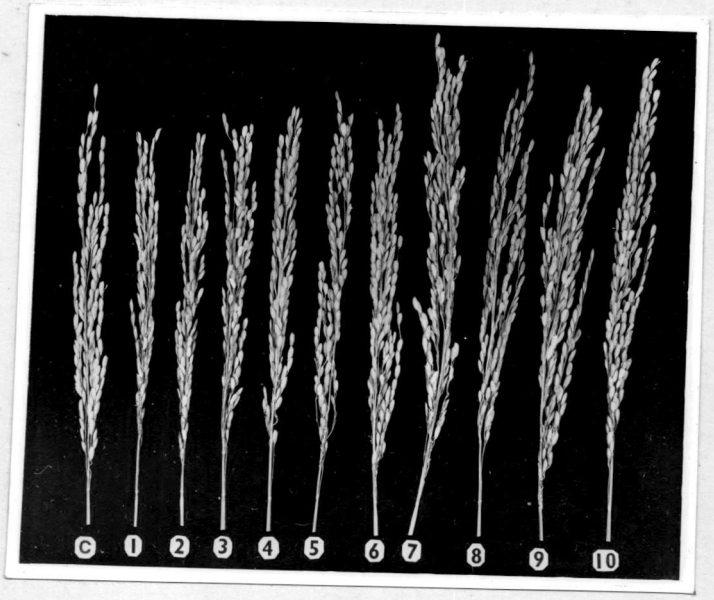


Figure 21

Figure 22. Ears of macro-mutants- III.

C. Control

1 to 10. Induced by EMS.

Figure 23. Ears of macro-mutants - IV.

C. Control.

1 to 10. Induced by NMU.

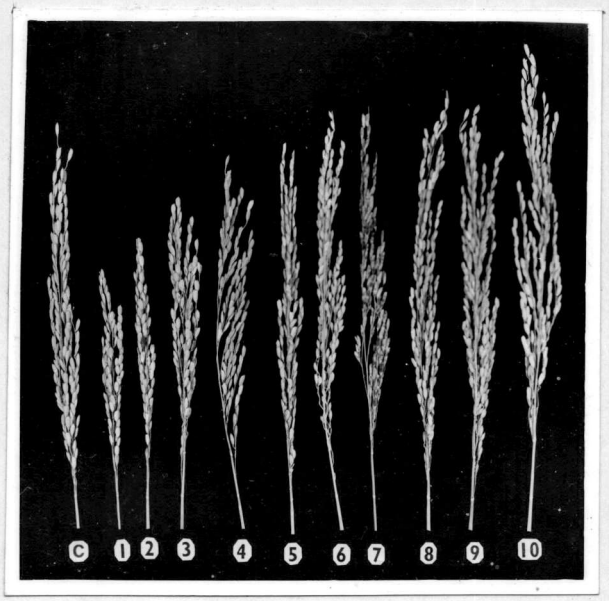


Figure 22

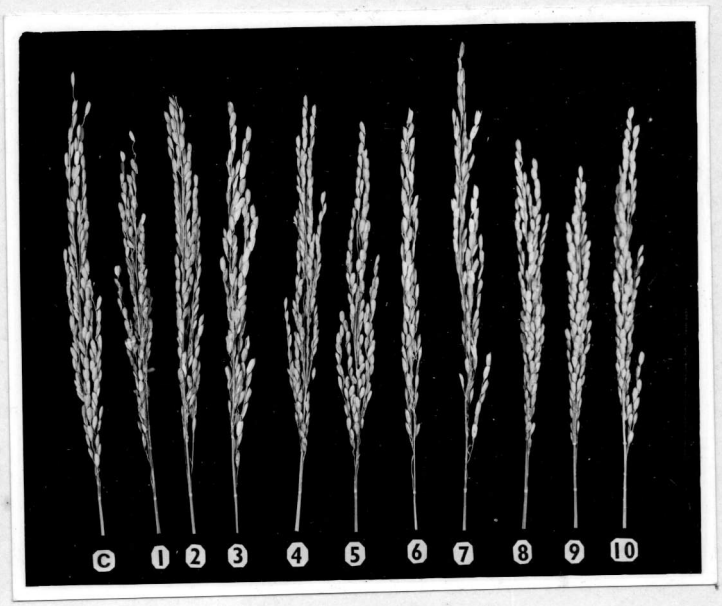


Figure 23

Figure 24. Grains of macro-mutants.

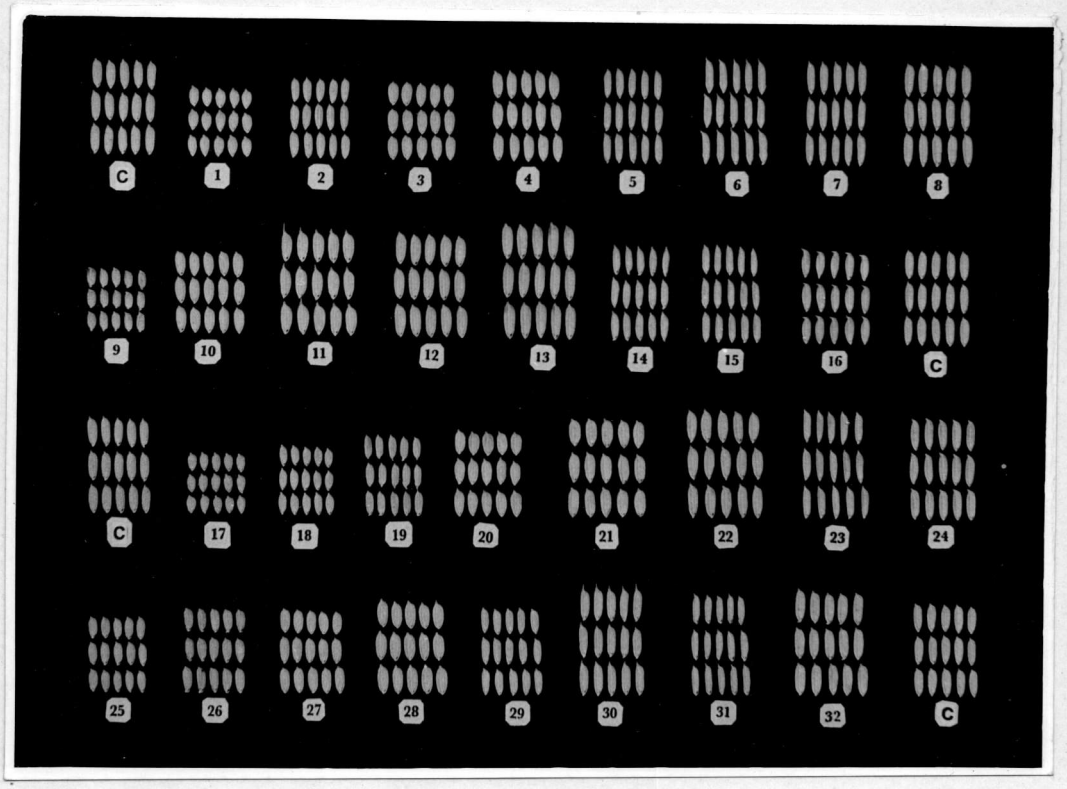
C. Control.

Nos. 1 to 8. Induced by gamma rays.

Nos. 9 to 16. Induced by fast neutrons.

Nos. 17 to 24. Induced by EMS.

Nos. 25 to 32. Induced by NMU.


C
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
C
C
17
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19
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21
22
23
24
25
26
27
28
29
30
31
32
C

Figure 24

Figure 25. Macro-mutants with awned grains - Ears.

C. Control.

Nos. 1 to 4. Induced by gamma rays.

Nos. 5 and 6. Induced by fast neutrons.

Nos. 7 to 11. Induced by EMS.

Figure 26. Macro-mutants with awned grains - Grains

C. Control.

Nos. 1 and 2. Induced by fast neutrons.

Nos. 3 to 5. Induced by EMS.

Nos. 6 to 11. Induced by gamma rays.

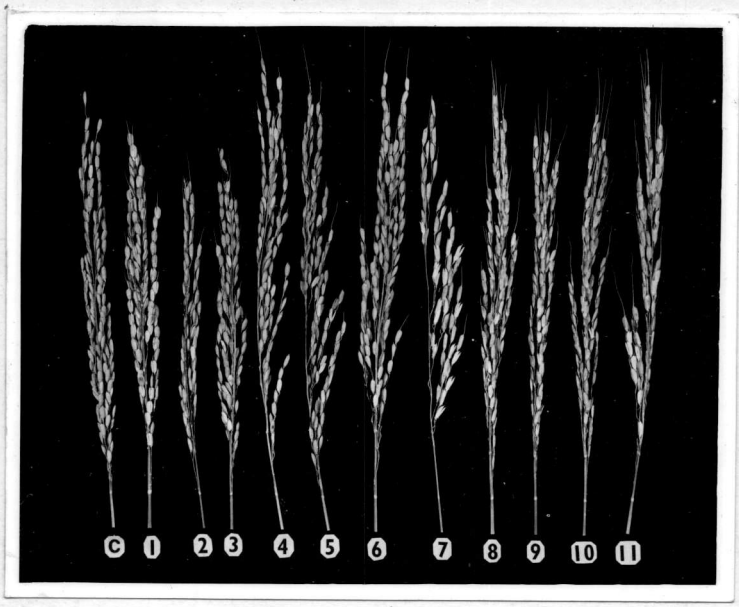


Figure 25

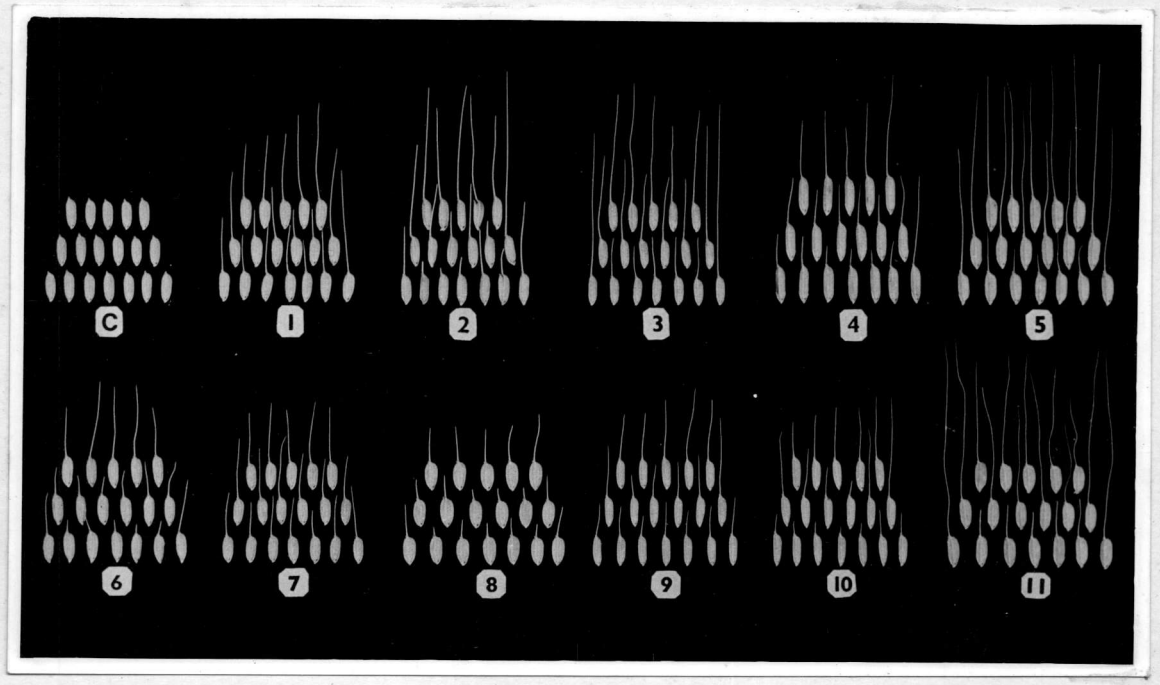


Figure 26

Figure 27. Tall mutants - I.

C. Control.

1. Induced by gamma rays.

2 and 3. Induced by EMS.

Figure 28. Tall mutants - II.

C. Control.

4. Induced by gamma rays.

5 and 6. Induced by EMS.

Figure 27

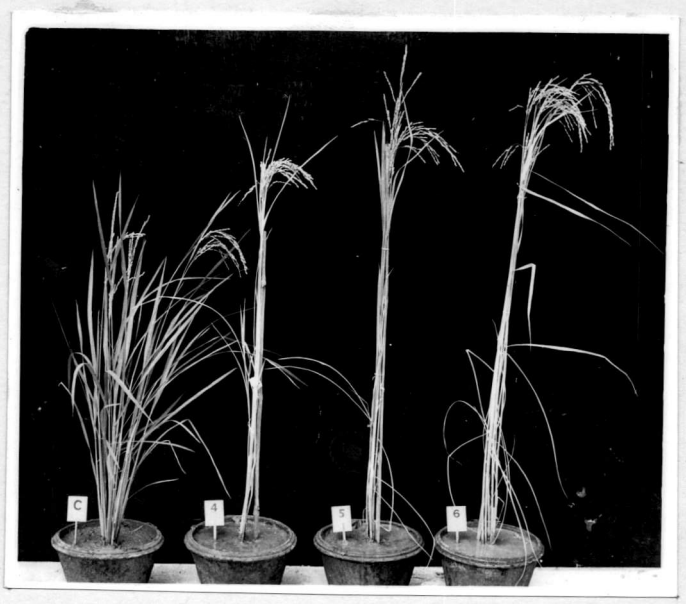


Figure 28

Figure 29. Early mutants.

C. Control.

1. Induced by gamma rays.

2 and 3. Induced by EMS.

Figure 30. Late mutants - I.

1. Control.

2. Induced by NMU.

3. Induced by EMS.

4. Induced by gamma rays.

Figure 31. Late mutants - II.

C. Control.

1 and 3. Induced by gamma rays.

2. Induced by EMS.

Figure 29

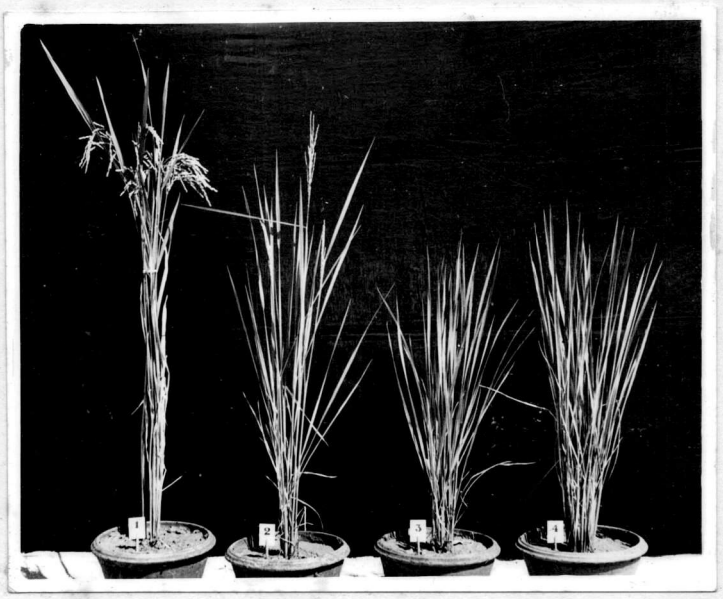


Figure 30

Figure 31

ones (Figures 30 and 31). There was a predominance of late types. The magnitude of lateness in late mutants was more than that of earliness in the early mutants. The shortest and the longest duration mutants had maturity periods of 85 and 180 days respectively in relation to 120 days of the parent variety. A long duration mutant with 180 days maintained its duration irrespective of changes in time of sowing thereby indicating that it was photo-insensitive. Mutants with changes in leaf character were mostly narrow leaved types (Figures 32 and 33). Ear type mutants were relatively rare and consisted of long ear (Figure 34) and compact ear types. Mutants with grain size differences were represented by small, medium or large grain types and also by those with bold or fine grains. Grain colour variations included mutants with brown, yellow brown, brown mottled, grey spotted and ash coloured hulls.

Several mutants (39 Nos.) with abnormal panicles (Figure 35), spikelets (Figures 36 and 37) or panicles and spikelets (Figures 38 and 39) were recorded. Visible mutants also included types (8 Nos.) with shattering grains, yellow internodes, sterile spikelets and others.

3. Systematic mutants

Mutants possessing characters which were not seen among the varieties of Oryza sativa were included under this category. Three mutants of this type were isolated. Two of them were tall and resembled varieties included under O.perennis subsp. barthii in many respects except the rhizomatous nature (Figure 40). The third one was dwarf and

Figure 32. Narrow leaved mutants - I.

1. Control.

2 and 3. Induced by fast neutrons.

4. Induced by EMS.

Figure 33. Narrow leaved mutant - II.

C. Control.

1. Induced by gamma rays.

Figure 32

Figure 33

Figure 34. Long ear mutants - Ears.

C. Control.

1 to 3. Induced by gamma rays.

4 and 5. Induced by fast neutrons.

6 and 7. Induced by EMS.

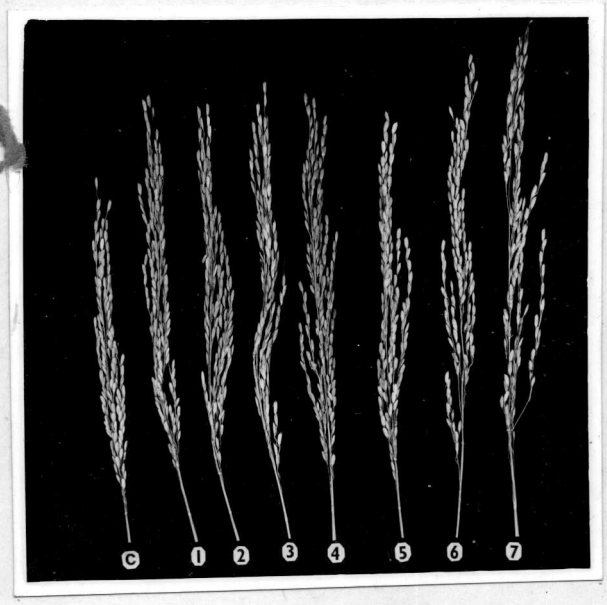


Figure 34

Figure 35. Abnormal panicle mutants - Ears.

C. Control.
1. Twisted rachis - fast neutrons
2. Reduced spikelets in upper half of panicle - fast neutrons.
3. Nonbranching panicle - fast neutrons.
4. Tip sterile - fast neutrons.
5. Neck leaf - EMS .
6. Sparse grains - EMS.
7. Fisty panicle - NMU.
8. Reduced upper part of rachis - NMU.
9. Elongated upper part of rachis - NMU.

Figure 36. Abnormal spikelet mutants - Ears.

C. Control.
1. Reduced palea - fast neutrons.
2. Double ridged palea - fast neutrons.
3. Open spikelets - fast neutrons.
4. Curved lemma and reduced palea - EMS.
5. Reduced and sunken palea - EMS.
6. Reduced palea - EMS.
7. Short awned lemma and reduced palea - NMU.
8. Beaked lemma and depressed palea - NMU.
9. Semiopen spikelets - NMU.

Figure 35

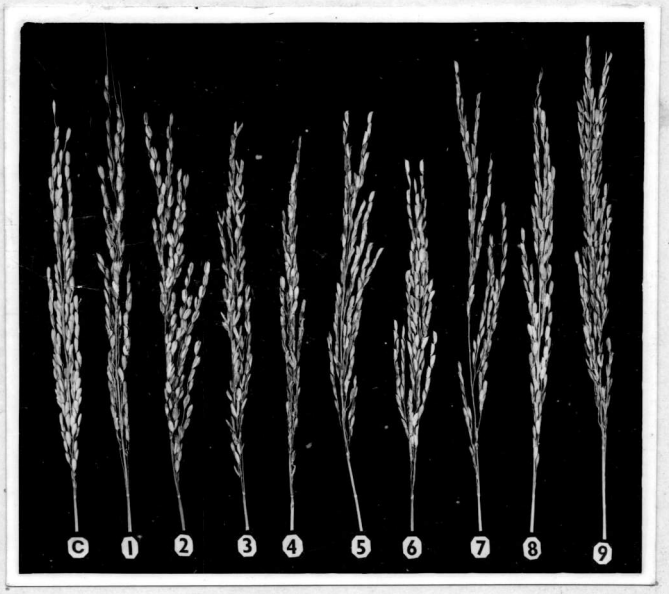


Figure 36

Figure 37. Abnormal spikelet mutants - grains.

C. Control.

1 to 9. Sterile grains.

1. Open glumes - fast neutrons.
2. Curved glumes - fast neutrons.
3. Reduced lemma - fast neutrons.
4. Reduced spikelet - fast neutrons.
5. Elongated lemma - NMU.
6. Curved glumes - NMU.
7. Open narrow glumes - EMS.
8. Reduced palea - EMS.
9. Open glumes - NMU.

10 to 17. Fertile grains

10. Reduced palea - EMS.
11. Beaked lemma - EMS.
12. Reduced palea - EMS.
13. Double ridged palea - EMS.
14. Reduced palea - NMU.
15. Short palea - NMU.
16. Elongated sterile glumes - gamma rays.
17. Double ridged palea and open glumes - fast neutrons.

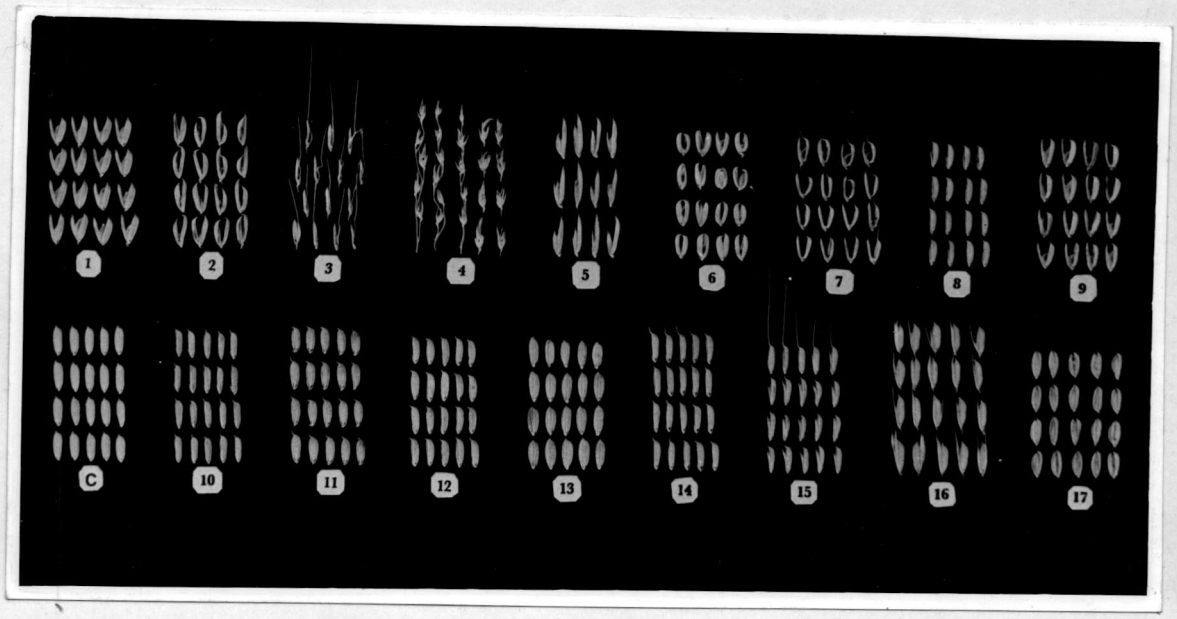
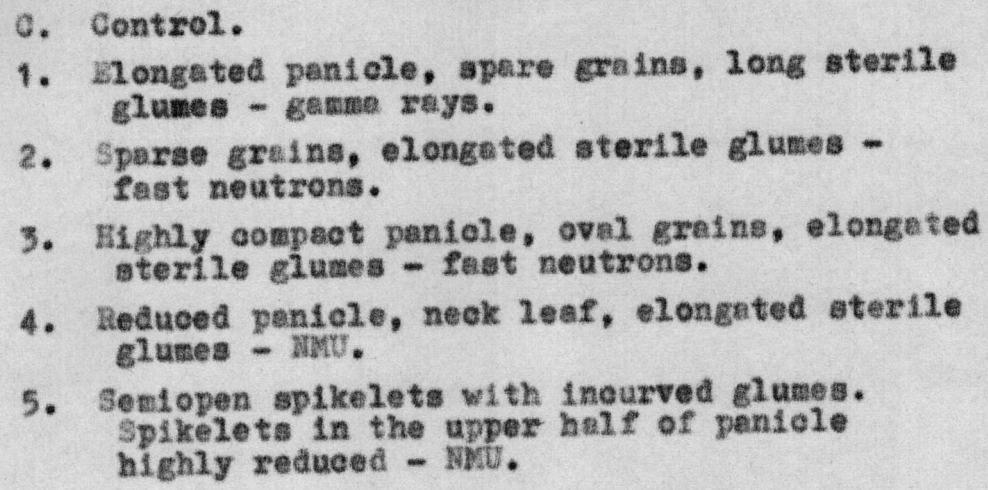


Figure 37

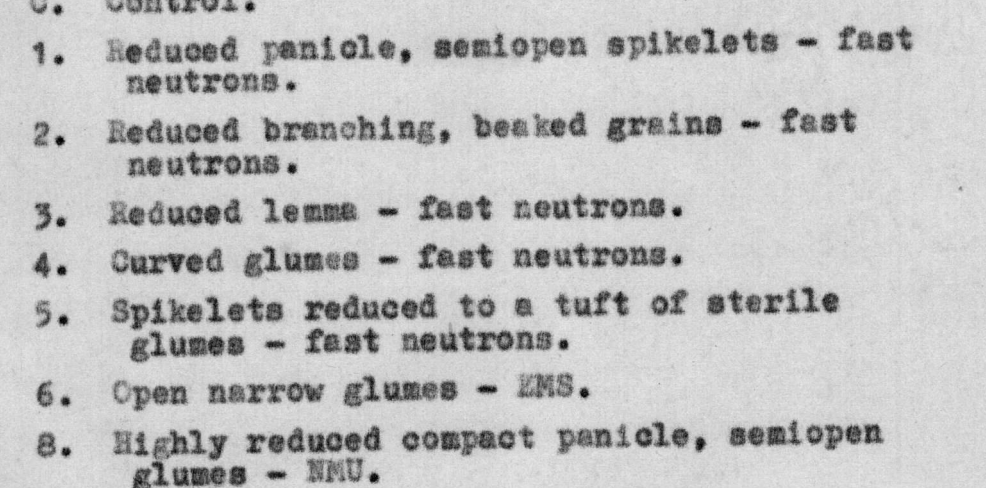
Figure 38. Abnormal panicle and spikelet mutants - Fertile.



C. Control.

1. Elongated panicle, spare grains, long sterile glumes - gamma rays.
2. Sparse grains, elongated sterile glumes - fast neutrons.
3. Highly compact panicle, oval grains, elongated sterile glumes - fast neutrons.
4. Reduced panicle, neck leaf, elongated sterile glumes - NMU.
5. Semiopen spikelets with incurved glumes. Spikelets in the upper half of panicle highly reduced - NMU.

Figure 39. Abnormal panicle and spikelet mutants - Sterile.



C. Control.

1. Reduced panicle, semiopen spikelets - fast neutrons.
2. Reduced branching, beaked grains - fast neutrons.
3. Reduced lemma - fast neutrons.
4. Curved glumes - fast neutrons.
5. Spikelets reduced to a tuft of sterile glumes - fast neutrons.
6. Open narrow glumes - EMS.
8. Highly reduced compact panicle, semiopen glumes - NMU.
9. Elongated lemma - NMU.
10. Open glumes - NMU.

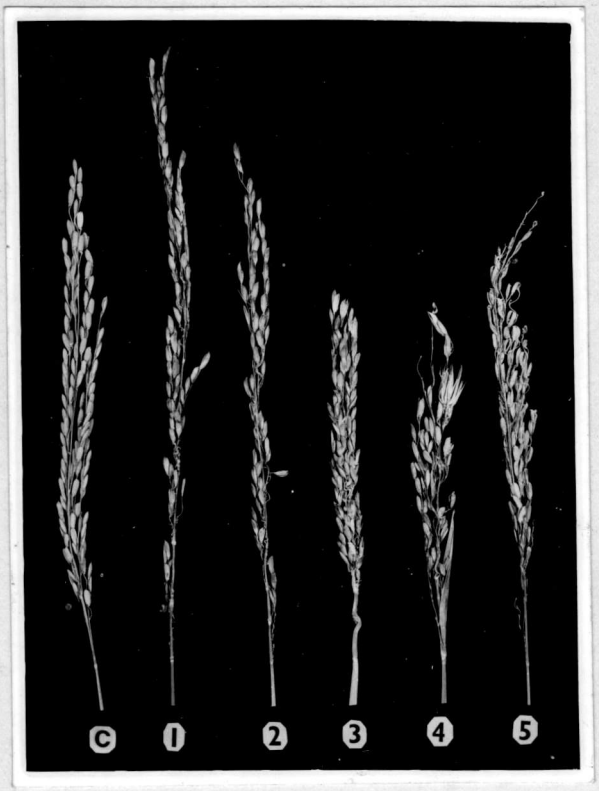


Figure 38

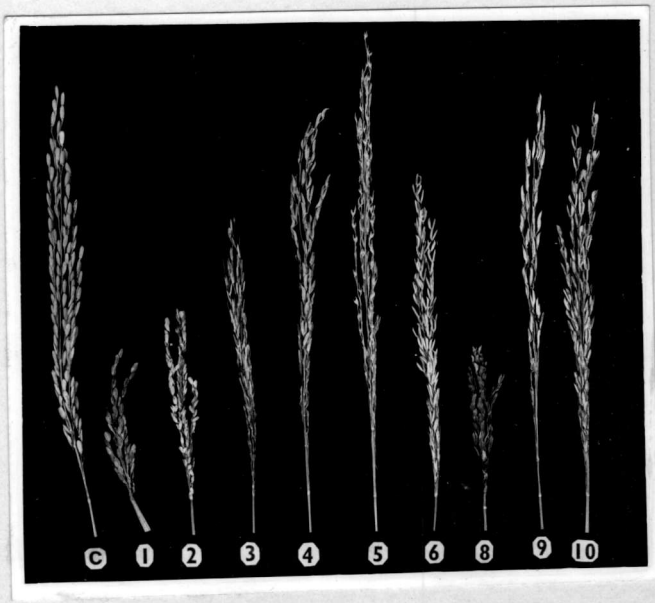


Figure 39

Figure 40. Systematic mutants.

1. Control.
2. Thick culm, few tillers, long leaves - also Fig. 41(3) - gamma rays.
3. Tall, thick culm, semiopen tillers, broad, coarse dark green leaves - also Fig. 41(2) - EMS.

Figure 41. Systematic mutants - Ears.

C. Control.
1. Short open panicle, small medium awned grains - EMS.
2. Partly enclosed long panicle. Big, hairy, medium awned spikelets. Highly sterile - EMS (same as Fig. 40-3).
3. Exerted compact panicle. Big, bold, hairy long awned spikelets. Semi sterile - gamma rays (same as Fig. 40-2).

Figure 40

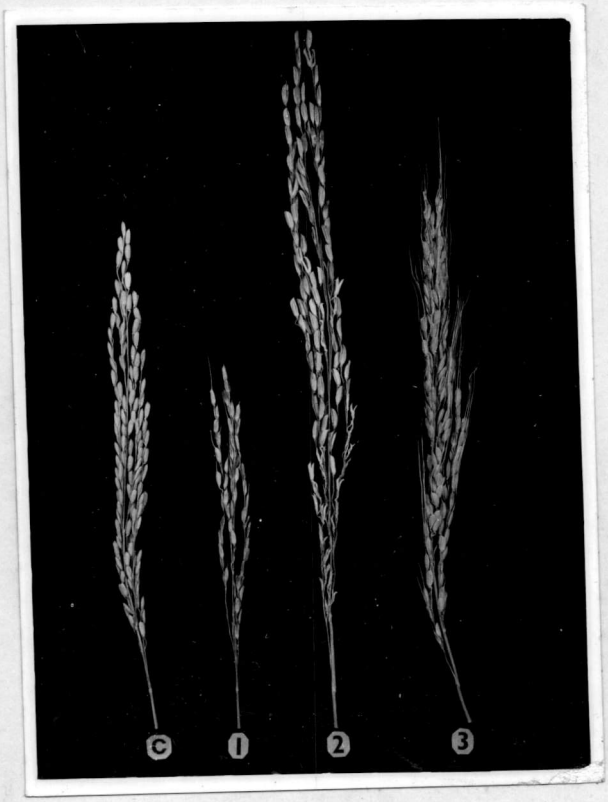


Figure 41

its ear resembled O. sativa v. fatua. The ear and grain characters of these mutants showed drastic differences from the parent variety (Figure 41). The mutants resembling japonica varieties in grain type were not classified under this category since they have been included under the mutants for grain type.

iv) Multiple mutations

The mean number of viable mutations per mutated ear increased with increasing doses of mutagens (Table XIII). The increase was most conspicuous in treatment with EMS. The high doses of gamma rays and fast neutrons also led to significant increase in the mean number of mutations per ear. This increase could be taken as an indication of the induction of multiple mutations that is, more than one mutation in the same ear. The frequencies and relative percentages of ears segregating for two and three types of mutations are also presented in Table XIII. The relative percentages of ears yielding more than one type of mutation increased with increasing doses of EMS, gamma rays and fast neutrons and the increase was most significant in treatment with EMS. Three cases of true multiple viable mutations and one of multiple chlorophyll and viable mutations were observed in treatments with EMS and NMU.

d. Total mutations

The total mutation frequencies presented as mutations per 100 M_1 spikes in Table XI increased with increasing doses of mutagens. In treatment with radiations there was a

TABLE XIII

Frequency and percentage of M_1 ear progenies segregating for single and multiple viable mutations

Mutagen and dose	No. of ear progenies segregating	Viable mutations: Total No.	Viable mutations: Mean No. per ear	Ear progenies segregating for mutations of: Frequency: One type	Two types	Three types	Relative %: One type	Two types	Three types
i) Gamma rays									
10 krad	13	14	1.08	12	1	..	92	8	..
20 ,,	30	36	1.20	24	6	..	80	20	..
30 ,,	33	37	1.12	29	4	..	88	12	..
40 ,,	32	38	1.19	26	6	..	81	19	..
50 ,,	17	23	1.35	13	2	2	76	12	12
Total	125	148	1.18	104	19	2	83	15	2
ii) Fast neutrons									
705 rad	8	10	1.25	6	2	..	75	25	..
968 ,,	15	18	1.20	12	3	..	80	20	..
1170 ,,	16	19	1.19	13	3	..	81	19	..
1408 ,,	16	18	1.13	14	2	..	88	12	..
1570 ,,	22	24	1.09	20	2	..	91	9	..
1710 ,,	19	25	1.32	13	6	..	68	32	..
1880 ,,	29	33	1.14	26	2	1	90	7	3
2100 ,,	15	17	1.13	13	2	..	89	11	..
Total	140	164	1.17	117	22	1	83	16	1
iii) EMS									
19 mM	2	2	1.00	2	..	..	100	..	..
38 ,,	4	4	1.00	4	..	..	100	..	..
58 ,,	10	12	1.20	8	2	..	80	20	..
77 ,,	8	9	1.13	7	1	..	88	12	..
96 ,,	11	16	1.45	7	3	1	64	27	9
115 ,,	17	21	1.23	13	4	..	76	24	..
154 ,,	24	30	1.25	18	6	..	75	25	..
192 ,,	30	37	1.23	24	5	1	80	17	3
240 ,,	37	46	1.24	29	7	1	78	19	3
288 ,,	41	53	1.29	30	10	1	73	24	3
Total	184	230	1.25	142	38	4	77	21	2
iv) NMU									
0.97 mM	13	13	1.00	13	..	..	100	..	..
1.94 ,,	18	18	1.00	18	..	..	100	..	..
2.91 ,,	13	14	1.08	12	1	..	92	8	..
3.88 ,,	18	20	1.11	16	2	..	89	11	..
4.85 ,,	15	15	1.00	15	..	..	100	..	..
5.82 ,,	29	29	1.00	29	..	..	100	..	..
7.76 ,,	19	20	1.05	18	1	..	95	5	..
9.70 ,,	17	17	1.00	17	..	..	100	..	..
Total	142	146	1.03	138	4	..	97	3	..

decrease in frequencies at the highest doses whereas, in treatment with chemical mutagens the increase was progressive upto the highest dose (Figure 42). The maximum frequency of total mutations was obtained with gamma rays followed by EMS, NMU and fast neutrons in decreasing order.

e. Interrelation of chlorophyll and viable mutations

The chlorophyll, viable and total mutation frequencies estimated as the number of mutations per 100 M_1 spikes are compared in Table XIV and graphically represented in Figure 42. Following treatment with fast neutrons, EMS and NMU the chlorophyll and viable mutation frequencies were almost similar, whereas with gamma rays the viable mutation frequencies were higher than the chlorophyll mutation frequencies. In treatments with gamma rays, fast neutrons and NMU, the total mutation frequencies were nearly the sum total of chlorophyll and viable mutation frequencies. This was also the case at the lower doses of EMS, whereas at higher doses the total mutation frequencies were less than the total of the chlorophyll and viable mutation frequencies.

The interrelation of ear-progenies segregating for chlorophyll and viable mutations was estimated and presented in Table XV. The frequencies of mutations against each mutagen indicated in this table were the sum of frequencies over all doses. The expected frequencies of double (chlorophyll and viable) mutations were calculated on the assumption that chlorophyll and viable mutations occurred independently in each ear. These calculated values were more or less the same

TABLE XIV

Interrelation of different types of mutations in the M_2 generation

Mutagen and dose	Mutations per 100 M_1 spikes (ears)			Viable/ chloro-phyll muta-tions	Viable/ total muta-tions
	Chloro-phyll	Viable	Total		
i) Gamma rays					
10 krad	11.3	11.5	21.2	1.0	0.54
20 ,,	8.0	29.4	35.3	3.7	0.83
30 ,,	6.8	36.3	44.0	5.3	0.82
40 ,,	24.3	49.2	63.1	2.0	0.78
50 ,,	5.6	47.2	50.0	8.4	0.94
ii) Fast neutrons					
705 rad	10.1	6.7	16.8	0.7	0.40
968 ,,	8.8	12.6	20.2	1.4	0.62
1170 ,,	12.5	13.3	24.2	1.1	0.55
1408 ,,	13.8	13.3	27.7	1.0	0.48
1570 ,,	12.1	18.5	26.1	1.5	0.71
1710 ,,	14.2	16.5	32.2	1.2	0.51
1880 ,,	15.8	25.0	36.2	1.6	0.69
2100 ,,	12.7	13.2	23.7	1.0	0.58
iii) EMS					
19 mM	5.0	1.7	7.6	0.3	0.22
38 ,,	6.7	3.4	11.0	0.5	0.31
58 ,,	12.3	8.3	18.3	0.7	0.45
77 ,,	9.0	6.8	17.8	0.8	0.38
96 ,,	15.4	9.4	23.1	0.6	0.41
115 ,,	11.2	14.3	26.1	1.3	0.55
154 ,,	20.3	20.3	33.1	1.0	0.61
192 ,,	22.8	25.0	45.0	1.1	0.56
240 ,,	22.3	31.3	43.2	1.4	0.72
288 ,,	32.5	34.7	51.7	1.1	0.67
iv) NMU					
0.97 mM	10.7	11.0	19.5	1.0	0.56
1.94 ,,	13.3	15.0	26.7	1.1	0.56
2.91 ,,	14.1	11.0	22.9	0.8	0.48
3.88 ,,	13.1	15.4	26.5	1.2	0.58
4.85 ,,	10.8	12.6	23.5	1.2	0.54
5.82 ,,	18.9	24.2	43.3	1.3	0.56
7.76 ,,	29.5	16.8	42.5	0.6	0.40
9.70 ,,	22.6	32.1	49.1	1.4	0.65

TABLE XV

Interrelation of ear progenies segregating for chlorophyll and viable mutations

Mutagen	No. of M_1 ear progenies scored	No. of M_1 ear progenies segregating for:				
		Chlorophyll mutations	Viable mutations	Chlorophyll or viable mutation	Both chlorophyll and viable mutations	
					Observed	Expected*
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i) Gamma rays	407	53	125	159	19	16.3
ii) Fast neutrons	941	127	140	243	24	18.9
iii) NMU	878	145	142	267	20	23.5
iv) EMS	1184	201	184	328	57	31.2
EMS - doses						
19 mM	118	7	2	9	0	0.1
38 "	118	9	4	13	0	0.3
58 "	120	15	10	22	3	1.2
77 "	118	15	8	21	2	1.0
96 "	117	17	11	27	1	1.6
115 "	119	15	17	31	1	2.1
154 "	118	21	24	39	6	4.3
192 "	120	33	30	54	9	8.3
240 "	118	27	37	51	13	8.5
288 "	118	42	41	61	22	14.6

* Expected on the assumption of independent occurance of chlorophyll and viable mutations in ears

$$(\text{Col. } 7 = \frac{\text{Col.3} \times \text{Col.4}}{\text{Col.2}})$$

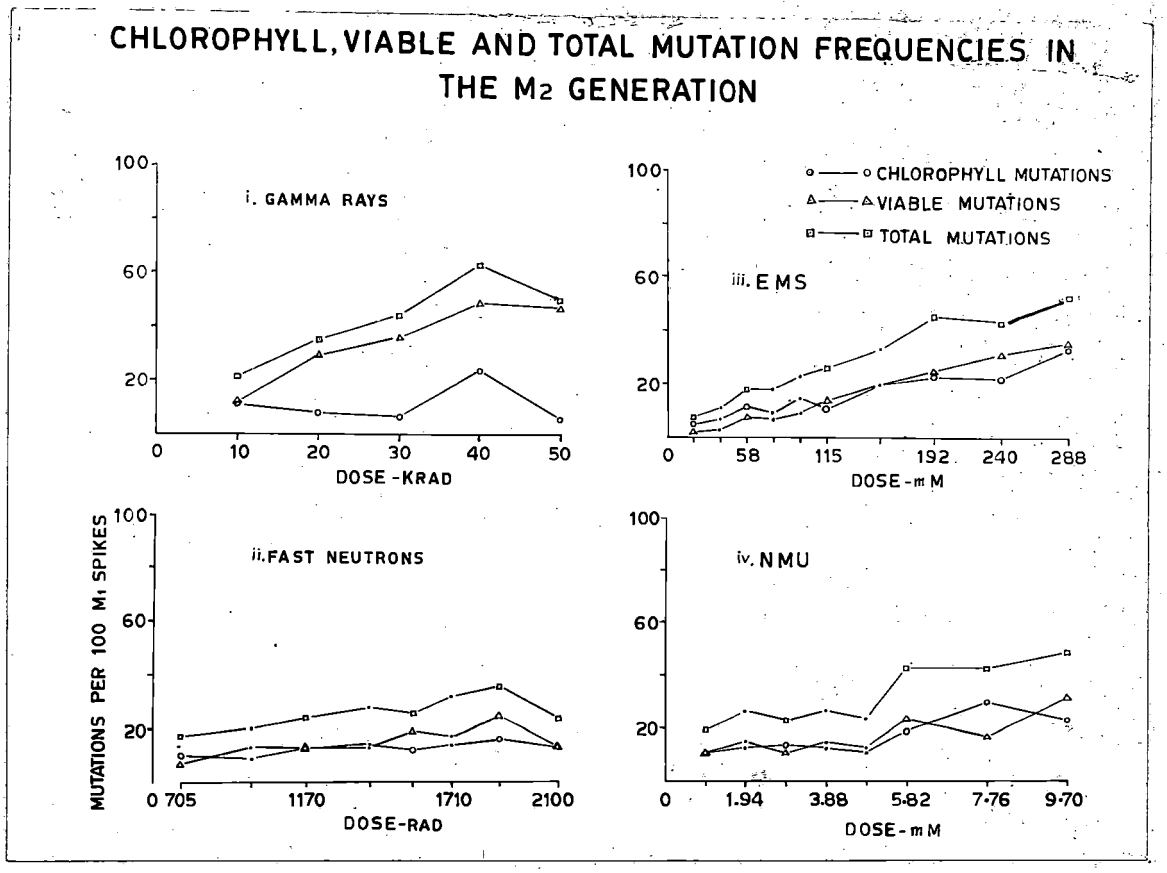

CHLOROPHYLL, VIABLE AND TOTAL MUTATION FREQUENCIES IN THE M2 GENERATION
i. GAMMA RAYS
DOSE - KRAD
ii. FAST NEUTRONS
DOSE-RAD
iii. EMS
DOSE - m M
iv. NMU
DOSE - m M
CHLOROPHYLL MUTATIONS
VIABLE MUTATIONS
TOTAL MUTATIONS
MUTATIONS PER 100 M1 SPIKES

Figure 42

as the observed frequencies of ears with double mutations in treatments with gamma rays, fast neutrons and NMU. On the other hand, in treatment with EMS, the observed frequencies of double mutations were much higher than the expected ones which indicated that EMS induced the two types of mutations concurrently at least in certain of the ear primordia. A dose-wise analysis indicated that the interrelation was most pronounced at the high doses.

f. Mutagenic effectiveness and efficiency

The effectiveness and efficiency of mutagens in inducing chlorophyll and viable mutations were estimated and presented in Tables XVI and XVII.

i) Chlorophyll mutations

Effectiveness decreased progressively with increasing doses of radiations and chemical mutagens (Table XVI). This indicated that the increase in the frequency of mutations was not proportional to the increase in the dose of the mutagen. Fast neutrons among radiations and NMU among the chemical mutagens were the most effective. NMU was several times more effective than EMS.

Mutagenic efficiency was also found to decrease with increasing doses of radiations, NMU and MNNG, irrespective of whether the criteria adopted for estimation was lethality, injury or sterility (Table XVI). EMS, MMS and DES also yielded similar results when efficiency was estimated on the basis of lethality alone. Efficiency estimates based on injury and sterility, on the other hand, were found to increase

TABLE XVI

Mutagenic effectiveness and efficiency (chlorophyll mutations)

Mutagen and dose	% survival reduction at 30 days (Lethality) L	% height reduction at 30 days (injury) =I	% seed fertility reduction (sterility) S	Mutations per 100 M$_1$ spikes (ears) (M)	Effectiveness Mx100 to or krad	Efficiency Mx100 L	Efficiency Mx100 I	Efficiency Mx100 S
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
i) Gamma rays								
10 krad	0	5	26.9	11.3	113	∝	226	42
20 ,,	0	9	57.3	6.0	40	∝	89	14
30 ,,	3	21	81.0	6.8	23	227	32	8
40 ,,	37	49	80.5	24.3	61	66	50	30
50 ,,	79	64	87.8	5.6	11	7	9	6
ii) X-rays								
5.0 krad	0	0	26.1	8.0	160	∝	∝	31
7.5 ,,	0	1	28.7	10.8	144	∝	1080	37
10.0 ,,	0	5	34.0	11.2	112	∝	224	33
12.5 ,,	0	6	39.0	11.6	93	∝	193	30
15.0 ,,	0	6	50.7	14.8	99	∝	247	29
17.5 ,,	0	6	51.2	14.9	85	∝	248	29
20.0 ,,	3	8	54.1	13.3	67	443	166	25
22.5 ,,	1	9	60.0	15.7	70	1570	174	26
25.0 ,,	3	10	62.7	11.2	45	373	112	18
30.0 ,,	2	13	63.5	15.0	50	750	115	23
35.0 ,,	7	19	64.2	16.6	47	237	87	26
40.0 ,,	26	27	70.1	14.8	37	57	55	21
iii) Fast neutrons								
705 rad	0	5	25.4	10.1	1433	∝	202	40
968 ,,	2	7	42.5	8.8	909	440	126	22
1170 ,,	2	8	39.6	12.5	1068	625	156	31
1408 ,,	1	10	44.8	13.8	980	1380	138	31
1570 ,,	2	13	53.5	12.1	771	605	93	23
1710 ,,	1	16	53.2	14.2	830	1420	89	27
1880 ,,	1	17	55.7	15.8	835	1580	93	28
2100 ,,	2	17	63.8	12.7	605	635	75	20
iv) DES								
11.4 mM	7	4	9.9	3.6	8	51	90	36
22.8 ,,	13	4	10.9	3.1	3	24	78	28
34.2 ,,	21	3	11.7	2.0	1	10	67	17
45.6 ,,	30	4	12.2	4.2	2	14	105	34
57.0 ,,	29	5	12.4	8.9	4	31	178	72

TABLE XVI (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
v) EMS								
19 mM	0	4	6.5	5.0	3.3	∞	125	77
38 "	3	5	7.3	6.7	2.2	223	134	92
58 "	4	9	10.0	12.3	2.7	307	137	123
77 "	3	9	9.7	9.0	1.5	300	100	92
96 "	6	14	12.0	15.4	2.0	257	110	128
115 "	11	13	11.7	11.2	1.2	102	86	96
154 "	12	15	13.4	20.3	1.6	169	135	151
192 "	11	15	17.3	22.8	1.5	207	152	132
240 "	12	16	28.8	22.3	1.2	186	139	77
288 "	11	17	30.4	32.5	1.4	295	191	107
336 "	15	16	30.7	27.3	1.0	182	171	89
384 "	17	19	33.9	26.5	0.9	156	140	78
vi) MMS								
1.2 mM	4	1	8.9	0.0	0.0	00	00	0
2.4 "	2	5	7.9	0.6	3.1	30	12	8
3.6 "	1	8	7.0	0.6	2.1	60	8	9
4.8 "	1	7	8.9	1.1	2.9	110	15	12
5.9 "	3	9	7.3	3.9	8.3	130	43	53
7.1 "	2	9	5.1	3.9	6.8	195	43	76
8.3 "	2	9	7.0	6.5	9.8	325	72	93
9.5 "	13	11	7.5	2.5	3.3	19	23	33
11.8 "	18	12	11.8	4.0	4.2	22	33	34
14.2 "	28	13	9.5	4.5	4.0	16	35	47
17.7 "	27	15	9.3	4.5	3.2	17	30	48
23.6 "	28	16	12.9	6.5	3.4	23	41	50
29.5 "	30	20	13.2	8.6	3.6	29	43	65
vii) NMU								
0.97 mM	2	12	5.8	10.7	137	535	89	183
1.94 "	21	24	4.0	13.3	86	63	55	333
2.91 "	47	29	4.5	14.1	61	30	49	313
3.88 "	54	32	4.0	13.1	42	24	41	328
4.85 "	56	43	3.8	10.8	28	19	25	284
5.82 "	65	50	4.2	18.9	41	29	34	450
7.76 "	76	55	8.0	29.5	47	39	54	369
9.70 "	78	59	9.7	22.6	29	29	38	231
viii) MNNG								
0.68 mM	0	3	1.7	1.7	31	∞	57	100
1.36 "	2	7	1.2	2.8	26	140	40	233
2.72 "	2	9	0.4	1.7	8	85	19	425
4.08 "	8	11	0.3	2.8	9	35	25	933
5.44 "	21	14	1.1	3.4	8	16	24	309
6.80 "	34	15	2.0	5.1	9	15	34	255
8.16 "	52	17	1.0	1.1	2	2	6	110
10.20 "	65	21	1.2	0.6	1	1	3	50

with increasing doses of EES and MMS but were more or less the same at the different doses of EMS. Fast neutrons among radiations and EMS among chemical mutagens were the most efficient.

ii) Viable mutations

Effectiveness of mutagens in inducing viable mutations was also found to be dose dependent (Table XVII). With increase in the dose the values decreased in the case of gamma rays and NMU, increased in the case of EMS and remained almost the same with fast neutrons. Fast neutrons among radiations and NMU among chemical mutagens were, thus, the most effective.

Efficiency estimates also showed a similar tendency to decrease with increasing doses of radiations and NMU irrespective of the basis for estimation (Table XVII). In treatments with EMS the efficiency increased with increasing doses. The rate of increase, however, was not proportional to the dose. Fast neutrons among radiations and EMS among chemical mutagens were the most efficient.

g. Potency of mutagens

The maximum frequencies of chlorophyll, viable and total mutations induced by the different mutagens are presented in Table XVIII. The maximum frequencies were induced at doses lower than the highest by most of the mutagens. Therefore, the dose range employed in the present study covered the effective doses. The mutagens could be ranked as follows in the order of their potency in inducing the highest frequency

TABLE XVII

Mutagenic effectiveness and efficiency (viable mutations)

Mutagen and dose	Lethality (L)	Injury (I)	Sterility (S)	Mutations per 100 M_1 spikes (ears) (M)	Effectiveness Mx100 to or krad	Efficiency Mx100/L	Efficiency Mx100/I	Efficiency Mx100/S
i) Gamma rays								
✓10 krad	0	5	26.9	11.5	115	∞	230	43
✓20 "	0	9	57.3	29.4	147	∞	327	51
✓30 "	3	21	81.0	36.3	121	1210	173	45
✓40 "	37	49	80.5	49.2	123	133	100	61
✓50 "	79	64	87.8	47.2	94	60	74	54
ii) Fast neutrons								
✓ 705 rad	0	5	25.4	6.7	953	∞	134	26
968 "	2	7	42.5	12.6	1302	630	180	30
✓1170 "	2	8	39.6	13.3	1137	665	166	34
✓1408 "	1	10	44.8	13.3	944	1330	133	30
1570 "	2	13	53.5	18.5	1178	925	142	34
✓1710 "	1	16	53.2	16.5	965	1650	103	31
1680 "	1	17	55.7	25.0	1329	2500	147	45
✓2100 "	2	17	63.8	13.2	629	660	77	21
iii) EMS								
19 mM	0	4	6.5	1.7	1.1	∞	43	26
38 "	3	5	7.3	3.4	1.1	113	68	45
✓ 58 "	4	9	10.0	8.3	1.8	208	92	83
77 "	5	9	9.7	6.8	1.1	227	76	70
96 "	6	14	12.0	9.4	1.2	157	67	78
✓115 "	11	13	11.7	14.5	1.6	130	110	122
154 "	12	15	13.4	20.3	1.6	169	135	151
✓192 "	11	15	17.3	25.0	1.6	227	167	145
✓240 "	12	16	28.8	31.3	1.6	261	196	108
✓288 "	11	17	30.4	34.7	1.7	315	204	114
iv) NMU								
0.97 mM	2	12	5.8	11.0	142	55	92	190
✓ 1.94 "	21	24	4.0	15.0	97	71	63	375
2.91 "	47	29	4.5	11.0	47	23	38	244
✓3.88 "	54	32	4.0	15.4	49	29	48	385
4.85 "	56	43	5.8	12.6	32	23	29	332
✓5.82 "	65	50	4.2	24.2	52	37	48	576
✓7.76 "	76	55	8.0	16.8	27	22	31	210
✓9.70 "	78	59	9.7	32.1	41	41	54	331

TABLE XVIII

Maximum frequencies of mutations induced by treatment of seeds with radiations and chemical mutagens

Mutagen	Dose	M_1 damage (% of control)			M_2 mutation frequency		
		Survival reduction (Lethality)	Height reduction (Injury)	Fertility reduction (Sterility)	Mutations per 100 M_1		Mutants per 100 M_2 seedlings
					Plants	Spikes (ears)	
A. Chlorophyll mutations							
i) Gamma rays	40 krad	37	49	80.5	25.4	24.3	5.31
ii) X-rays	40 ,,	26	27	70.1	23.3	14.8	3.44
iii) Fast neutrons	1680 rad	1	17	55.7	27.5	15.8	2.52
iv) EMS	288.00 mM	11	17	30.4	48.0	32.5	3.57
v) MMS	29.50 ,,	30	20	13.2	13.0	8.6	0.51
vi) NMU	7.76 ,,	76	55	8.0	42.1	29.5	3.00
vii) MNNG	5.44 ,,	21	14	1.1	5.6	3.4	0.40
viii) DES	57.00 ,,	29	5	12.4	13.4	8.9	0.40
B. Vieble mutations							
i) Gamma rays	40 krad	37	49	80.5	NE	49.2	NE*
ii) Fast neutrons	1680 rad	1	17	55.7	NE	25.0	NE
iii) EMS	288.00 mM	11	17	30.4	NE	34.7	NE
iv) NMU	9.7 ,,	78	59	9.7	NE	32.1	NE
C. Total mutations							
i) Gamma rays	40 krad	37	49	80.5	NE	63.1	NE
ii) Fast neutrons	1680 rad	1	17	55.7	NE	36.2	NE
iii) EMS	288.00 mM	11	17	30.4	NE	51.7	NE
iv) NMU	9.7 ,,	78	59	9.7	NE	49.1	NE

*NE - Not estimated

of chlorophyll mutations.

M_1 spike basis:

EMS > NMU > Gamma rays > Fast neutrons > X-rays

M_2 plant basis:

Gamma rays > EMS > X-rays > NMU > Fast neutrons

The shift in the relative positions of gamma rays and X-rays to higher ranks when mutation frequency was estimated on M_2 plant basis was because of the increase in segregation ratios consequent on an increase in the size of the mutated sector. The ranking of mutagens in relation to viable and total mutation frequency was;

Gamma rays > EMS > NMU > Fast neutrons.

Thus, gamma rays and EMS induced very high mutation frequencies.

The frequencies of mutations induced by different mutagens at doses producing similar degrees of M_1 damage are presented in Table XIX. At LD_{20} for injury and LD_{50} for sterility, fast neutrons induced more chlorophyll mutations than gamma rays, whereas gamma rays induced more viable mutations than fast neutrons. At LD_{20} for lethality and injury, EMS induced more chlorophyll and viable mutations than NMU, whereas at LD_{10} for sterility NMU induced more chlorophyll and viable mutations than EMS. This inverse relation was because of the very low effect of NMU on the fertility of M_1 plants. Both radiations and chemical mutagens could be compared only at one level of M_1 damage viz., LD_{20} for injury and the mutagens could be ranked in the

TABLE XIX

Frequencies of mutations induced by radiations and chemical mutagens at similar levels of M_1 damage

Mutagen	Dose	Chlorophyll mutations: Mutations per 100 M_1 plants	Chlorophyll mutations: Mutations per 100 M_1 spikes (ears)	Chlorophyll mutations: Mutants per 100 M_2 plants	Viable mutations per 100 M_1 spikes (ears)	Total mutations per 100 M_1 spikes (ears)
A. Radiations						
1) At LD.20 for injury (height reduction)						
Gamma rays	30 krad	9.0	6.8	1.19	36.3	44.0
X-rays	35 ,,	25.0	16.6	3.33	NS	NS*
Fast neutrons	1880 rad	27.5	15.8	2.52	25.0	36.2
ii) At LD.50 for sterility						
Gamma rays	20 krad	14.4	8.0	1.19	29.4	35.3
X-rays	20 ,,	23.6	13.3	1.52	NS	NS
Fast neutrons	1880 rad	27.5	15.8	2.52	25.0	36.2
B. Chemicals						
i) At LD.20 for lethality (Survival reduction)						
EMS	384.00 mM	40.9	26.5	3.14	>34.7	>51.7
MMS	11.80 ,,	8.0	4.0	0.53	NS	NS
NMU	1.94 ,,	22.2	13.3	1.14	15.0	26.7
MNNG	5.44 ,,	5.6	3.4	0.40	NS	NS
DES	34.20 ,,	3.9	2.0	0.10	NS	NS
ii) At LD.20 for injury						
EMS	384.00 mM	40.9	26.5	3.14	>34.7	>51.7
MMS	29.50 ,,	13.0	8.6	0.51	NS	NS
NMU	1.94 ,,	22.2	13.3	1.14	15.0	26.7
MNNG	10.20 ,,	1.1	0.6	0.01	NS	NS
DES	Not attained					
iii) At LD.10 for sterility						
EMS	96.0 mM	25.8	15.4	1.30	9.4	23.1
MMS	14.2 ,,	9.0	4.5	0.34	NS	NS
NMU	9.7 ,,	33.3	22.6	2.82	32.1	49.1
MNNG	Not attained					
DES	11.4 ,,	6.1	3.6	0.09	NS	NS

* NS. Not Studied

following order of their potency.

Chlorophyll mutations:

EMS > Fast neutrons > NMU > Gamma rays

Viable mutations:

Gamma rays > EMS > Fast neutrons > NMU

Total mutations:

EMS > Gamma rays > Fast neutrons > NMU

h. Relative biological effectiveness

RBE is the ratio of the doses of two or more radiations producing equal or similar effects in tissues. The RBE of gamma rays and fast neutrons estimated with X-rays as the standard for the different criteria for M_1 damage are presented in Table XX. The values for gamma rays in respect of the different criteria ranged from 0.5 to 1.3, thereby indicating that the biological effectiveness of X-rays and gamma rays was almost the same. On the other hand the RBE values offast neutrons ranged from 8 to 29 for the different criteria indicating its high biological effectiveness. RBE values were the lowest for root length and highest for germination.

i. Inheritance of M_1 chlorophyll chimeras

The M_1 plants with chlorophyll deficient sectors were studied in the M_2 generation on ear-progeny basis to understand the mode of inheritance of this induced abnormality. The results are presented in Table XXI. Sections 1 and 2 of this table indicated that the chlorophyll mutation

TABLE XX

Relative biological effectiveness (RBE) of radiations for various criteria in the M_1 generation

Criteria	Level of M_1 damage	Doses inducing similar effects			RBE		
		X-rays (krad)	Gamma rays (krad)	Fast neutrons (krad)	X-rays	Gamma rays	Fast neutrons
1) Germination on 4th day	LD.20	>40	60	> 2.10	..	1.0	< 29
2) Length of primary root on 4th day	LD.25	15	20-30	1.88	1.0	0.5-0.75	8.0
3) Length of coleoptile on 4th day	LD.20	25	30	2.10	1.0	0.8	11.9
4) Seedling height on 30th day	LD.20	35	30	1.88	1.0	1.2	18.6
5) Survival on 30th day	LD.25	40	30-40	> 2.10	1.0	1.0-1.3	<19
6) Seed fertility	LD.50	20	20	1.88	1.0	1.0	10.6

TABLE XXI

Interrelation of M_1 chlorophyll chimeras and M_2 chlorophyll mutations

	Fast neutrons	EMS	NMU	F.N. + NMU
1. Progenies of normal tillers from chimeric plants				
i) No.scored	20	56	120	125
ii) No.segregating	2	5	18	14
iii) % segregating	10.0	9.1	15.0	11.2
2. Progenies of chimeric tillers				
i) No. scored	9	52	81	86
ii) No. segregating	2	14	25	35
iii) % segregating	22.2	26.9	30.9	40.7
3. i) No.of chimeric tillers	9	52	81	86
ii) No. of chimeric tillers producing striped panicles	3	5	15	4
iii) % of chimeric tillers with striped panicles	33.3	9.6	18.5	4.7
4. Progenies of normal panicles on chimeric tillers				
i) No. scored	6	47	66	82
ii) No. segregating	2	13	12	32
iii) % segregating	33.3	27.7	18.2	39.0
5. Progenies of striped panicles on chimeric tillers				
i) No. scored	3	5	15	4
ii) No. segregating	0	1	13	3
iii) % segregating	..	20.0	86.7	75.0
6. Progenies of normal spikelets on striped panicles				
i) No. scored	3	5	3	4
ii) No. segregating	0	1	2	1
iii) % segregating	..	20.0	66.7	25.0
7. Progenies of striped spikelets				
i) No. scored	3	5	15	4
ii) No. segregating	0	0	13	2
iii) % segregating	..	..	86.7	50.0

frequency of chimeric tillers was two to four times of that of non-chimeric tillers on chimeric plants. This indicated a positive association between M_1 chlorophyll chimeras and M_2 chlorophyll mutations. However, only 22 to 41 per cent of ears from chimeric tillers segregated for mutations indicating that the association was only partial. It was also observed that the chimeric tillers produced striped panicles only in a few cases (Table XXI-3). There were also a few cases of striped panicles borne on normal tillers. These results indicated that the chlorophyll deficient sectors on vegetative plant parts such as leaves and culm and on the reproductive parts such as the panicles and spikelets have independent origins.

Sections 4 and 5 of Table XXI further indicated that the striped panicles on chimeric tillers segregated for mutations more frequently than normal panicles on striped tillers. Therefore, when the panicles were also chimeric the possibility for them to contain mutations had further increased. The striped panicles invariably possessed sectors for striped and normal spikelets. The M_2 progeny from striped spikelets yielded chlorophyll mutants more frequently than those from the normal ones (Table XXI-6&7).

Two distinctly different patterns of striping were observed on the spikelets.

1) The sides of the spikelet were white and the dorsal and ventral ridges of the lemma and palea were green. This was the pattern of striping in striped spikelets on chimeric tillers obtained in treatments with fast neutrons, EMS and MNU.

2) The sides of the spikelets were green and the dorsal and ventral ridges of the lemma and palea were white. This was the pattern of striping in striped spikelets on normal tillers and were obtained only in treatment with NMU. Such spikelets were highly sterile.

There was a strong association between the pattern of striping on the spikelets and the appearance of the progeny. Striped spikelets of the first type (white on sides) segregated giving a few chlorophyll deficient seedlings, whereas those of the second type (green on sides) always gave only albino seedlings in the progeny. The results therefore, revealed that the "green on sides" type of albino striping on the spikelets was an indication of albinism in the embryo of the seeds derived therefrom.

j. M_1 fertility and M_2 chlorophyll mutations

The chlorophyll mutation frequency estimated as mutations per 100 M_1 spikes and mutants per 100 M_2 seedlings in each of the fertility classes in the different doses of gamma rays and EMS are given in Table XXII. The frequencies are arranged in the two way table (Table XXIII) to reveal the interrelation of M_1 fertility and M_2 mutation frequency. The number of mutations per 100 M_1 spikes increased progressively with decreasing fertility up to the fertility class of 21 to 40 per cent. When the frequency was estimated as number of mutants per 100 M_2 seedlings a similar trend was noticed with gamma rays. With EMS the frequencies increased progressively

TABLE XXII

Frequency of chlorophyll mutations in the M_2 generation in the different M_1 seed fertility classes

Mutagen and dose	% of seed fertility in the M_1 ear	No. of M_1 ear progenies		No. of M_2		Mutations per 100 M_1 spikes (ears)	Mutants per 100 M_2 seedlings
		Scored	Segregating	Seedlings	Chlorophyll mutants		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1) Gamma rays							
10 krad	>80	152	3	9110	12	2.0	0.13
	61-80	160	10	7814	43	6.3	0.55
	41-60	127	10	3755	31	7.9	0.83
	21-40	24	1	374	2	4.1	0.53
	<20	..	..	..	..	..	..
	Pooled	463	24	21053	88	5.2	0.42
20 krad	>80	49	4	2350	28	8.2	1.19
	61-80	100	10	4691	52	10.0	1.11
	41-60	176	8	5379	39	4.5	0.72
	21-40	74	4	1124	15	5.4	1.16
	<20	58	..	373	..	..	..
	Pooled	457	26	13917	132	5.7	0.94
30 krad	>80	15	3	586	13	20.0	2.22
	61-80	27	1	1116	9	3.7	0.81
	41-60	142	7	4610	26	4.9	0.56
	21-40	126	9	2170	30	7.1	1.38
	<20	73	4	440	5	5.5	1.14
	Pooled	383	24	8922	83	6.3	0.93
40 krad	>80	18	..	912	..	..	..
	61-80	13	2	624	7	14.6	1.12
	41-60	75	16	2251	87	21.3	3.87
	21-40	90	29	1466	111	32.2	7.56
	<20	74	10	317	18	13.5	5.68
	Pooled	270	57	5572	223	21.1	4.00
50 krad	>80	..	..	..	..	..	..
	61-80	..	..	..	..	..	..
	41-60	38	2	1190	9	5.3	0.76
	21-40	43	2	928	6	4.7	0.65
	<20	25	..	160	..	..	..
	Pooled	106	4	2278	15	3.8	0.66

TABLE XXII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
11) EMS							
38 mM	>80	277	3	20744	39	1.1	0.19
	61-80	39	...	2147	..	..	..
	41-60	..	..	..	..	..	..
	21-40	..	..	..	..	..	..
	<20	..	..	..	..	..	..
	Pooled	316	3	22891	39	0.9	0.17
77 mM	>80	250	1	20271	1	0.4	0.01
	61-80	50	1	2652	1	2.0	0.04
	41-60	12	1	494	14	8.3	2.83
	21-40	..	..	..	..	..	..
	<20	..	..	..	..	..	..
	Pooled	312	3	23417	16	0.9	0.07
115 mM	>80	245	11	19768	106	4.5	0.54
	61-80	90	6	4572	53	6.7	1.16
	41-60	28	2	982	10	7.1	1.02
	21-40	..	..	..	..	..	..
	< 20	..	..	..	..	..	..
	Pooled	363	19	25322	169	5.2	0.67
154 mM	>80	80	13	4633	96	16.2	2.07
	61-80	45	9	2164	66	20.0	3.05
	41-60	55	16	1557	61	29.1	3.92
	21-40	26	9	431	19	34.6	4.41
	<20	45	4	298	6	8.9	2.01
	Pooled	251	51	9083	248	20.3	2.73
192 mM	>80	101	11	6195	63	10.9	1.02
	61-80	50	11	1781	63	22.0	3.54
	41-60	58	17	1441	65	29.3	4.51
	21-40	43	16	616	43	37.2	6.98
	<20	33	5	212	8	15.1	3.77
	Pooled	285	60	10245	242	21.1	2.36

TABLE XXII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
EMS (continued)							
240 mM	>80	100	9	6366	74	9.0	1.16
	61-80	66	12	2968	111	18.2	3.74
	41-60	67	14	1904	64	20.9	3.36
	21-40	54	11	684	28	20.4	4.09
	<20	54	13	252	20	24.1	7.94
	Pooled	341	59	12174	297	17.3	2.44
288 mM	>80	68	13	3926	145	19.1	3.69
	61-80	39	7	1502	46	17.9	3.06
	41-60	34	10	1084	47	29.4	4.34
	21-40	45	11	659	37	24.4	5.61
	<20	74	12	427	24	16.2	5.62
	Pooled	260	53	7598	299	20.4	3.93
336 mM	>80	270	25	17483	210	9.3	1.20
	61-80	165	31	7848	220	18.8	2.80
	41-60	99	35	3575	200	35.4	5.60
	21-40	65	26	1261	98	40.0	7.77
	<20	67	19	468	42	28.4	8.95
	Pooled	666	136	30635	770	20.4	2.51
384 mM	>80	132	11	6872	121	8.3	1.76
	61-80	202	25	10617	176	12.2	1.66
	41-60	150	40	5508	153	26.7	2.78
	21-40	95	33	1904	104	34.7	5.46
	<20	48	13	306	24	27.1	7.84
	Pooled	627	122	25207	578	19.5	2.29

TABLE XXIII

Interrelation of M_1 seed fertility and M_2 chlorophyll mutation frequency

(Dose wise in each mutagen)

Mutagen and dose	Mutation frequency in the different M_1 seed fertility classes. (% fertility)					
	>80	61-80	41-60	21-40	<20	Pooled
(i) Per 100 M1 spikes (ears)						
i) Gamma rays						
10 krad	2.0	6.3	7.9	4.1	..	5.2
20 ..	8.2	10.0	4.5	5.4	..	5.7
30 ..	20.0	3.7	4.9	7.1	5.5	6.3
40 ..	..	14.6	21.3	32.2	13.5	21.1
50 ..	..	..	5.3	4.7	..	3.8
ii) EMS						
38 mM	1.1	..	..	..	..	0.9
77 ..	0.4	2.0	8.3	..	..	0.9
115 ..	4.5	6.7	7.1	..	..	5.2
154 ..	16.2	20.0	29.1	34.6	8.9	20.3
192 ..	10.9	22.0	29.3	37.2	15.1	21.1
240 ..	9.0	18.2	20.9	20.4	24.1	17.3
288 ..	19.1	17.9	29.4	24.4	16.2	20.4
336 ..	9.3	18.8	35.4	40.0	28.4	20.4
384 ..	8.3	12.2	26.7	34.7	27.1	19.5
(ii) Per 100 M2 seedlings						
i) Gamma rays						
10 krad	0.13	0.55	0.83	0.53	..	0.42
20 ..	1.19	1.11	0.72	1.16	..	0.94
30 ..	2.22	0.81	0.56	1.38	1.14	0.93
40 ..	..	1.12	3.87	7.56	5.68	4.00
50 ..	..	..	0.76	0.65	..	0.66
ii) EMS						
38 mM	0.19	..	..	..	..	0.17
77 ..	0.01	0.04	2.83	..	..	0.07
115 ..	0.54	1.16	1.02	..	..	0.67
154 ..	2.07	3.05	3.92	4.41	2.01	2.73
192 ..	1.02	3.54	4.51	6.98	3.77	2.36
240 ..	1.16	3.74	3.36	4.09	7.94	2.44
288 ..	3.69	3.06	4.34	5.61	5.62	3.93
336 ..	1.20	2.80	5.60	7.77	8.95	2.51
384 ..	1.76	1.66	2.78	5.46	7.84	2.29

up to the lowest class of fertility viz., less than 20 per cent. The trend was the same when the frequencies were considered dose-wise in each mutagen (Table XXIII) and also when the doses were combined (Table XXIV, Figure 43-A). The percentages of mutants in mutated ears were found to increase with decreasing fertility in both gamma rays and EMS (Table XXIV).

The spectrum of chlorophyll mutants in the different fertility classes is presented in Table XXV. The frequencies are graphically represented in Figure 43-B. Albinas were predominant in the highest fertility class following treatment with gamma rays. In the next lower class its frequency was very low which had further increased with decreasing fertility. In treatment with EMS, the frequency of albinas decreased slightly with decreasing fertility. Xanthas progressively decreased with decreasing fertility in both the mutagens. The other types of mutations did not show any consistent pattern.

III. Attempts for enhancing mutagenic efficiency

a. Combination treatments

The data on survival and seedling height in the M_1 generation recorded in the two sets of combination treatments are presented in Table XXVI. Chlorophyll mutation frequencies in the M_2 generation estimated as mutations per 100 M_1 spikes and as mutants per 100 M_2 seedlings are presented in Table XXVII. The relative percentages of different types (spectrum) of chlorophyll mutants are presented in Table XXVIII. The M_1 and M_2 data are arranged in the two way table in order to assess

TABLE XXIV

Interrelation of M_1 seed fertility and M_2 chlorophyll mutation frequency (mutagen wise - doses combined)

Seed fertility of M_1 ear (%)	Mean No. of seedlings per ear progeny	No. of M_1 ear progenies		No. of M_2 -		Mutations per 100 M_1 spiked (ears)	Mutants per 100 M_2 seedlings	% of mutants in mutated ears
		Scored	Segregating	Seedlings	Chlorophyll mutants			
1) Gamma rays								
> 80	55	234	10	12958	53	4.3	0.41	11.7
61 - 80	47	300	23	14245	111	7.7	0.78	10.9
41 - 60	31	558	43	17185	192	7.7	1.12	14.7
21 - 40	17	357	45	6064	162	12.6	2.67	20.3
< 20	6	230	14	1290	23	6.1	1.78	30.7
11) EMS								
> 80	70	1523	97	106258	859	6.4	0.80	15.2
61 - 80	50	746	102	36251	736	13.7	2.03	16.7
41 - 60	33	503	135	16545	614	26.8	3.71	15.8
21 - 40	17	328	106	5555	329	32.3	5.92	17.8
< 20	6	321	66	1963	124	20.6	6.32	26.7

TABLE XXV

Relative percentages of different types (spectrum) of M_2 chlorophyll mutants in the different M_1 seed fertility classes

Seed fertility of M_1 ear (%)	Total No.of mutants	Types of chlorophyll mutants							
		A	X	V	C	AV	S	T	Others*
1) Gamma rays									
>80	53	73	23	..	4	..	..	..	..
61 - 80	111	27	26	9	26	12	..	..	..
41 - 60	192	34	11	23	21	11	..	..	..
21 - 40	162	58	2	14	16	8	2	..	..
<20	23	74	13	9	4	..	..	..	..
ii) EMS									
>80	855	42	9	19	11	15	4	..	..
61 - 80	736	47	1	17	23	10	1	..	1
41 - 60	614	36	3	29	17	11	1	2	1
21 - 40	329	36	6	26	18	8	4	..	2
<20	124	27	..	15	37	15	3	3	..

* K and EL

TABLE XXVI

Survival and seedling height in the M_1 generation in combination treatment of radiations and NMU

Mutagen		Survival (% of control)		Seedling height (% of control)	
Radiation dose	NMU dose	15th day	30th day	15th day	30th day
i) Fast neutrons + NMU					
Control		100	100	100	100
705 rad	"	100	100	93	95
968 rad	"	99	98	88	93
1170 rad	"	99	98	86	92
"	0.97 mM	97	96	85	90
"	1.94 "	83	80	81	80
"	2.91 "	61	60	76	72
"	3.88 "	51	48	71	69
705 rad	0.97 "	93	93	79	85
"	1.94 "	74	71	72	78
"	2.91 "	56	54	63	69
"	3.88 "	47	43	58	64
968 rad	0.97 "	93	91	72	78
"	1.94 "	65	64	65	74
"	2.91 "	64	61	60	67
"	3.88 "	46	45	56	62
1170 rad	0.97 "	96	94	68	77
"	1.94 "	63	61	61	70
"	2.91 "	55	53	57	66
"	3.88 "	45	44	53	61
ii) Gamma rays + NMU					
Control		100	100	100	100
10 krad	"	98	98	94	92
20 krad	"	100	99	83	88
30 krad	"	99	99	69	83
"	0.48 mM	94	94	79	88
"	0.97 "	92	91	74	83
10 krad	0.48 "	94	94	71	84
"	0.97 "	90	90	66	79
20 krad	0.48 "	90	90	62	80
"	0.97 "	90	88	60	78
30 krad	0.48 "	92	91	58	77
"	0.97 "	90	86	48	70

TABLE XXVII

Chlorophyll mutation frequency in the M_2 generation in combination treatments of radiations and NMU

Mutagen		No. of M_1 ear progenies		No. of M_2		Mutations per 100 M_1 spikes (ears)	Mutants per 100 M_2 seedlings
Radiation dose	NMU dose	Scored	Segregating	Seedlings scored	Chlorophyll mutants		
1) Fast neutrons + NMU							
Control	..	348	0	24532	0	..	..
705 rad	..	338	32	16486	162	9.5	0.96
968 rad	..	314	31	13122	138	9.5	1.05
1170 rad	..	342	38	14076	151	11.1	1.07
..	0.97 mM	315	29	18197	167	9.2	0.92
..	1.94 ..	303	32	14342	188	10.6	1.31
..	2.91 ..	346	44	18643	250	12.7	1.34
..	3.88 ..	328	46	19186	288	14.0	1.50
705 rad	0.97 ..	370	32	16857	147	8.6	0.87
..	1.94 ..	332	45	12713	301	13.6	2.37
..	2.91 ..	308	54	12263	438	17.5	3.57
..	3.88 ..	273	71	10789	422	26.0	3.91
968 rad	0.97 ..	289	41	11385	268	14.2	2.35
..	1.94 ..	316	55	11000	393	17.4	3.57
..	2.91 ..	318	62	10369	327	19.5	3.15
..	3.88 ..	318	60	12710	411	18.8	3.23
1170 rad	0.97 ..	317	36	11866	145	11.4	1.22
..	1.94 ..	323	58	14359	422	18.0	2.94
..	2.91 ..	300	55	12749	390	18.3	3.06
..	3.88 ..	285	74	11636	458	26.0	3.94
11) Gamma rays + NMU							
Control		210	0	8718	0	..	..
10 krad	..	242	15	7889	51	6.2	0.64
20 krad	..	246	18	5615	73	7.3	1.30
30 krad	..	196	18	4487	59	9.2	1.31
..	0.48 mM	103	6	4794	25	5.8	0.52
..	0.97 ..	102	9	4316	39	8.8	0.90
10 krad	0.48 ..	106	3	3967	14	2.8	0.35
..	0.97 ..	103	4	2913	18	3.9	0.62
20 krad	0.48 ..	89	5	1750	6	5.7	0.34
..	0.97 ..	80	6	1530	54	7.5	3.53
30 krad	0.48 ..	73	6	1237	19	8.2	1.54
..	0.97 ..	72	9	1114	32	12.5	2.87

TABLE XXVIII

Relative percentages of different types (spectrum) of chlorophyll mutants in the M_2 generation in combination treatments of radiations and NMU

Mutagen		Total No.of mutants	Relative percentages of chlorophyll mutants							
Radiation dose	NMU dose		A	X	V	C	AV	S	T	BL
1) Fast neutrons + NMU										
705 rad	..	162	26	12	22	23	14	3	..	..
968 rad	..	138	25	8	22	15	12	8	10	..
1170 rad	..	151	45	8	27	13	5	2	..	..
..	0.97 mM	167	49	15	34	1	..	1	..	..
..	1.94 ..	188	45	12	17	..	23	..	1	2
..	2.91 ..	250	28	16	27	9	6	1	1	12
..	3.88 ..	288	29	2	20	8	21	1	19	..
705 rad	0.97 ..	147	36	5	16	23	4	5	..	11
..	1.94 ..	301	31	7	44	10	7	..	1	..
..	2.91 ..	438	13	4	15	52	3	..	6	7
..	3.88 ..	422	33	2	19	5	22	..	13	6
968 rad	0.97 ..	268	27	23	11	13	7	17	..	2
..	1.94 ..	393	45	2	12	38	3	..	..	..
..	2.91 ..	327	49	..	26	7	11	6	..	1
..	3.88 ..	411	31	31	26	4	8	..	..	..
1170 rad	0.97 ..	145	22	7	32	22	16	1	..	..
..	1.94 ..	422	14	9	38	27	10	-	2	..
..	2.91 ..	390	23	3	10	45	13	1	..	7
..	3.88 ..	458	24	16	30	23	5	1	1	..
ii) Gamma rays + NMU										
10 krad	..	51	33	12	22	31	2	..	..	..
20 krad	..	73	39	..	23	..	37	1	..	..
30 krad	..	59	39	..	49	4	5	3	..	..
..	0.48 mM	25	..	..	20	8	64	8	..	..
..	0.97 ..	39	33	10	26	21	10	..	..	..
10 krad	0.48 ..	14	64	..	14	22	..	..	..	..
..	0.97 ..	18	..	..	22	28	..	50	..	..
20 krad	0.48 ..	6	66	..	17	17	..	..	..	..
..	0.97 ..	54	33	24	..	20	15	8	..	..
30 krad	0.48 ..	19	42	..	16	32	10	..	..	..
..	0.97 ..	32	41	..	..	59	..	..	..	..

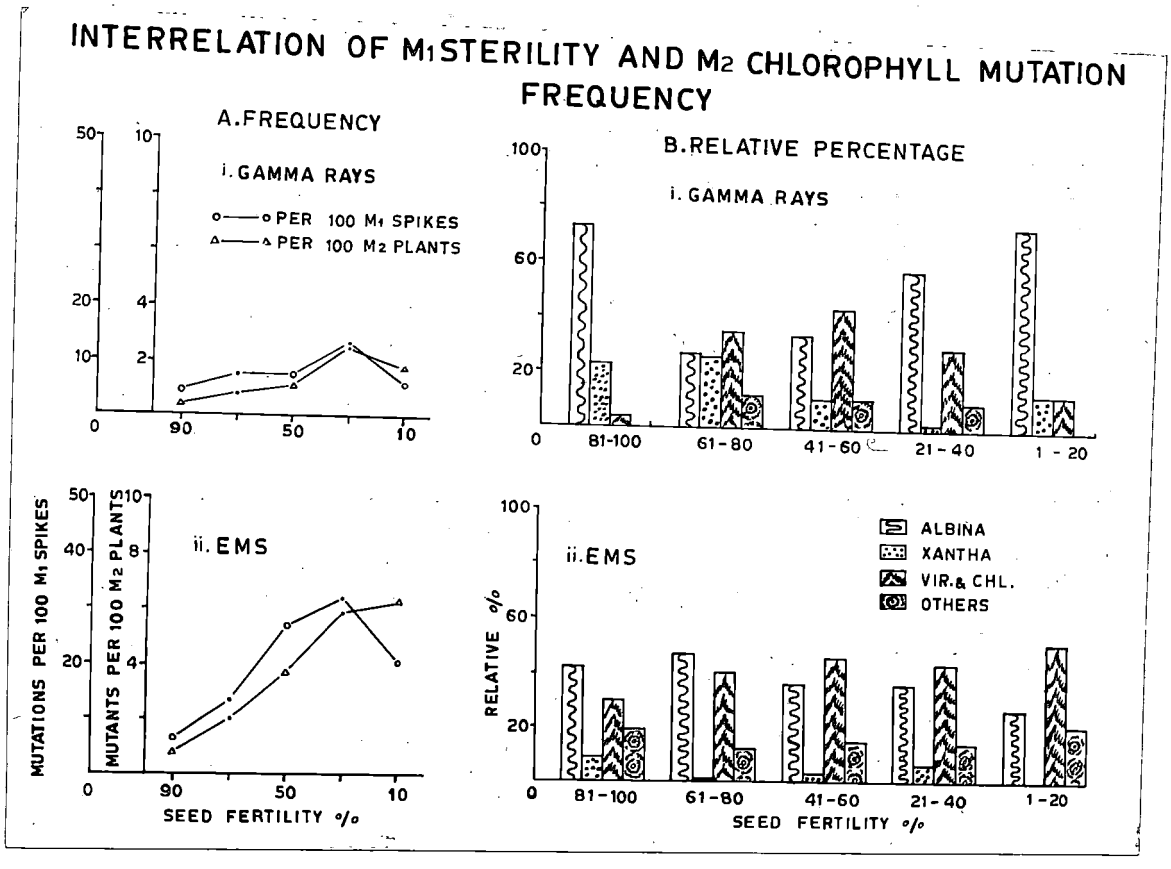

INTERRELATION OF M1 STERILITY AND M2 CHLOROPHYLL MUTATION FREQUENCY
A. FREQUENCY
i. GAMMA RAYS
PER 100 M1 SPIKES
PER 100 M2 PLANTS
B. RELATIVE PERCENTAGE
i. GAMMA RAYS
81-100
61-80
41-60
21-40
1-20
ii. EMS
MUTATIONS PER 100 M1 SPIKES
MUTANTS PER 100 M2 PLANTS
SEED FERTILITY %
ALBINA
XANTHA
VIR.& CHL.
OTHERS
RELATIVE %

Figure 43

the interaction effects of the two mutagens in the combined treatments (Table XXIX).

The percentages of survival and seedling height in either of the combination treatments did not differ significantly from the expected values based on additive effects (Table XXIX—iii).

The frequencies of chlorophyll mutations per 100 M_1 spikes in the combination of gamma rays and NMU were not higher than the values expected on the basis of additive effects. In the combination of fast neutrons and NMU, the frequencies were less than additive at the lower dose combinations, but at higher doses the observed frequencies were the same as the expected ones (Table XXIX-iii). The frequency of chlorophyll mutants estimated as the number of mutants per 100 M_2 seedlings revealed a different picture. In either of the combinations the observed mutant frequencies were significantly higher than the expected ones based on additive effects, at all dose combinations except at very low doses. This positive synergistic effect on mutant frequencies was most significant at the higher dose combinations of fast neutrons and NMU (Table XXIX-iv).

The percentages of mutants in mutated ears in combination treatments were significantly higher than those in individual treatments (Table XXIX-v). The segregation ratios at the higher dose combinations were considerably high indicating a strong positive synergistic effect. The increase in segregation ratio reflects a corresponding increase in the

TABLE XXIX

Interrelation of M_1 effects and M_2 chlorophyll mutation frequency in combination treatments of radiation and NMU

(i) Survival of seedlings - 30th day (% of control)

Radiation doses	NMU - Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	100	..	96	80	60	48
705 rad	100	..	93 (96)	71 (80)	54 (60)	43 (48)
968 rad	98	..	91 (93)	64 (78)	61 (59)	45 (47)
1170 rad	98	..	94 (93)	61 (78)	53 (59)	44 (47)
Gamma rays						
Control	100	94	91	..	..	..
10 krad	98	94 (92)	90 (89)	..	..	..
20 krad	99	90 (93)	88 (90)	..	..	..
30 krad	99	91 (93)	86 (90)	..	..	..

ii) Height of seedlings - 30th day (% of control)

Radiation doses	NMU - Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	100	..	90	80	72	69
705 rad	95	..	85 (86)	78 (76)	69 (68)	64 (66)
968 rad	93	..	78 (84)	74 (74)	67 (67)	62 (64)
1170 rad	92	..	77 (83)	70 (74)	66 (66)	61 (64)
Gamma rays						
Control	100	88	83	..	..	..
10 krad	92	84 (81)	79 (76)	..	..	..
20 krad	88	80 (77)	78 (73)	..	..	..
30 krad	83	77 (73)	70 (69)	..	..	..

Expected percentages estimated on the basis of additive effects for the combined treatments are given in brackets below.

TABLE XXIX (CONTD.)

iii) Mutations per 100 M_1 spikes (ears)

Radiation doses	NMU – Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	0	..	9.2	10.6	12.7	14.0
705 rad	9.5	..	8.6 (18.7)	13.6 (20.1)	17.5 (22.2)	26.0 (23.5)
968 rad	9.9	..	14.2 (19.1)	17.4 (20.5)	19.5 (22.6)	18.8 (23.9)
1170 rad	11.1	..	11.4 (20.3)	18.0 (21.7)	18.3 (23.8)	26.0 (25.1)
Gamma rays						
Control	0	5.8	8.8	..	..	..
10 krad	6.2	2.8 (12.0)	3.9 (15.0)	..	..	..
20 krad	7.3	5.7 (13.1)	7.5 (16.1)	..	..	..
30 krad	9.2	8.2 (15.0)	12.5 (18.0)	..	..	..

iv) Mutants per 100 M_2 seedlings

Radiation doses	NMU – Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	0	..	0.92	1.31	1.34	1.90
705 rad	0.96	..	0.87 (1.88)	2.37 (2.27)	3.57 (2.30)	3.91 (2.46)
968 rad	1.05	..	2.35 (1.97)	3.57 (2.36)	3.15 (2.39)	3.23 (2.55)
1170 rad	1.07	..	1.22 (1.99)	2.94 (2.38)	3.06 (2.41)	3.94 (2.57)
Gamma rays						
Control	0	0.52	0.90	..	..	..
10 krad	0.64	0.35 (1.16)	0.62 (1.54)	..	..	..
20 krad	1.30	0.34 (1.82)	3.53 (2.20)	..	..	..
30 krad	1.31	1.54 (1.83)	2.87 (2.21)	..	..	..

TABLE XXIX (CONTD.)

v) Percentage of mutants in mutated ears

Radiation doses	NMU – Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	0	..	13.9	13.6	12.6	13.0
705 rad	11.0	..	12.6	19.8	20.7	17.4
968 ,,	10.4	..	16.4	19.2	18.3	19.6
1170 ,,	8.5	..	12.6	16.0	18.9	16.0
Gamma rays						
Control	0	8.5	12.8	..	..	..
10 krad	11.0	16.3	16.5	..	..	..
20 ,,	10.2	4.6	38.3	..	..	..
30 ,,	11.8	13.2	18.9	..	..	..

vi) Relative percentage of albina mutants

Radiation doses	NMU – Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	0	..	49	45	28	29
705 rad	26	..	36	31	15	33
968 ,,	25	..	27	45	49	31
1170 ,,	45	..	22	14	23	24
Gamma rays						
Control	0	0	33	..	..	..
10 krad	33	64	0	..	..	..
20 ,,	39	66	33	..	..	..
30 ,,	39	42	41	..	..	..

vii) Relative percentage of viridis mutants

Radiation doses	NMU – Doses (mM)					
	0	0.48	0.97	1.94	2.91	3.88
Fast neutrons						
Control	0	..	34	17	27	20
705 rad	22	..	16	44	15	19
968 ,,	22	..	11	12	26	26
1170 ,,	27	..	32	38	10	30
Gamma rays						
Control	0	20	26	..	..	..
10 krad	22	14	22	..	..	..
20 ,,	23	17	0	..	..	..
30 ,,	49	16	0	..	..	..

size of the mutated sector.

The relative percentages of albina and viridis mutants were not influenced by combination treatments (Table XXIX-viiivii). The spectrum of chlorophyll mutants mostly remained unaltered (Table XXVIII).

b. Recurrent and alternate treatments

The data on survival and seedling height in the M_2^2 generation and chlorophyll mutation frequency in the M_3^2 generation from two sets of experiments viz., recurrent and alternate mutagen treatments in successive generations are presented in Tables XXX and XXXI respectively. For evaluating the mutagenic effects of the second treatment in the successive generation, the data are arranged in a two way table (Table XXXII).

Seedling survival percentages in the M_2^2 generation did not differ from the values estimated in relation to the respective M_2 values in any of the repeated mutagen treatments (Table XXXII-i). Seedling height in recurrent irradiation also showed the same trend, whereas in alternate treatments the M_2^2 values were slightly higher than those estimated from the concerned M_2 values (Table XXXII-ii).

The frequencies of mutations per 100 M_2^2 spikes are presented as section (iii) in Table XXXII. The observed frequencies in recurrent as well as alternate treatments were less than the sum total of frequencies in the respective controls in most of the cases. The lower mutation frequencies indicated that the mutagenic effect of the same or different

TABLE XXI

Survival and seedling height in the second generation (M_2^2) in repeated mutagen treatments

Mutagen and dose		Survival (% of control)		Seedling height (% of control)	
First treatment	Second treatment	15th day	30th day	15th day	30th day
1) Recurrent treatment					
Gamma rays	Gamma rays				
Control	"	100	100	100	100
"	10 krad	98	98	94	92
"	20 "	100	99	83	88
"	30 "	99	99	69	82
10 krad	"	96	96	101	99
"	10 krad	98	98	93	95
"	20 "	98	97	79	88
"	30 "	98	96	63	79
20 krad	"	97	96	101	97
"	10 krad	100	96	89	91
"	20 "	98	97	79	87
"	30 "	97	94	61	77
30 krad	"	95	92	100	93
"	10 krad	97	94	94	94
"	20 "	95	94	81	86
"	30 "	97	96	59	73
11) Alternate treatment					
Gamma rays	NMU				
"	0.97 mM	96	95	79	90
"	1.94 "	88	78	77	74
"	2.91 "	72	57	68	70
10 krad	0.97 "	95	95	78	90
"	1.94 "	85	73	70	84
"	2.91 "	73	58	65	81
20 krad	0.97 "	94	94	84	88
"	1.94 "	84	73	70	81
"	2.91 "	80	57	62	74
30 krad	0.97 "	89	88	80	89
"	1.94 "	84	74	73	84
"	2.91 "	79	57	59	75

TABLE XXXI

Chlorophyll mutation frequency in the third generation (M_3^2) in repeated mutagen treatments

Mutagen and dose		No. of M_3^2 ear progenies		No. of M_3^2 -		Mutations per 100 M_2^2 spikes (ears)	Mutants per 100 M_3^2 seedlings
First treatment	Second treatment	Scored	Segregating	Seedlings	Chlorophyll mutants		
i) Recurrent treatment							
Gamma rays	**Gamma rays**						
Control	..	210	0	8718	0	..	..
..	10 krad	242	15	7889	51	6.2	0.64
..	20 ,,	246	18	5615	73	7.3	1.30
..	30 ,,	196	18	4487	59	9.2	1.31
10 krad	..	229	5	9471	16	2.2	0.17
,,	10 krad	259	21	7649	118	8.2	1.54
,,	20 ,,	210	17	4343	71	8.1	1.63
,,	30 ,,	162	19	3551	103	11.7	2.90
20 krad	..	246	6	10497	36	2.4	0.34
,,	10 krad	214	12	6252	25	5.6	0.40
,,	20 ,,	205	13	3739	37	6.3	1.00
,,	30 ,,	161	14	3105	79	8.7	2.54
30 krad	..	227	13	8030	76	5.7	0.94
,,	10 krad	224	19	6121	79	8.4	1.29
,,	20 krad	162	13	3897	77	8.0	1.98
,,	30 ,,	152	18	2644	100	11.6	3.78
ii) Alternate treatment							
Gamma rays	**NMU**						
..	0.97 mM	182	6	9925	44	5.3	0.44
..	1.94 ,,	163	6	8540	31	3.7	0.36
..	2.91 ,,	123	8	7045	42	6.5	0.60
10 krad	0.97 ,,	216	9	10123	39	4.2	0.39
,,	1.94 ,,	188	4	8544	15	2.1	0.17
,,	2.91 ,,	155	7	7002	25	4.5	0.36
20 krad	0.97 ,,	235	16	10385	89	6.8	0.86
,,	1.94 ,,	192	14	9691	85	7.3	0.88
,,	2.91 ,,	184	19	9058	114	10.3	1.26
30 krad	0.97 ,,	204	17	8203	83	8.3	1.01
,,	1.94 ,,	188	13	7598	89	6.9	1.17
,,	2.91 ,,	172	22	6619	103	12.8	1.56

TABLE XXXII

Cumulative action for M_2^2 effects and M_3^2 chlorophyll mutation frequency in repeated mutagen treatments

(i) Survival of seedlings - 30th day (M_2^2) (% of the double control)

Gamma rays	Control	Recurrent-Gamma rays			Alternate - NMU		
		10 krad	20 krad	30 krad	0.97 mM	1.94 mM	2.91 mM
Control	100	98	99	99	95	78	57
10 krad	96	98	97	96	95	73	58
		(94)*	(95)	(95)	(91)	(75)	(55)
20 krad	96	96	97	94	94	73	57
		(94)	(95)	(95)	(91)	(75)	(55)
30 krad	92	94	94	96	88	74	57
		(90)	(91)	(91)	(87)	(72)	(52)

(ii) Height of seedlings - 30th day (M_2^2) (% of the double control)

Gamma rays	Control	Recurrent-Gamma rays			Alternate - NMU		
		10 krad	20 krad	30 krad	0.97 mM	1.94 mM	2.91 mM
Control	100	92	88	82	90	74	70
10 krad	99	95	88	79	90	84	81
		(91)*	(87)	(81)	(89)	(73)	(69)
20 krad	97	91	87	77	88	81	74
		(89)	(85)	(79)	(87)	(72)	(68)
30 krad	93	94	86	73	89	84	75
		(86)	(82)	(76)	(84)	(69)	(65)

(iii) Mutation frequency (per 100 M_2^2 ears)

Gamma rays	Control	Recurrent-Gamma rays			Alternate - NMU		
		10 krad	20 krad	30 krad	0.97 mM	1.94 mM	2.91 mM
Control	..	6.2	7.3	9.2	3.3	3.7	6.5
10 krad	2.2	6.2	8.1	11.7	4.2	2.1	4.5
		(8.4)**	(9.5)	(11.4)	(5.5)	(5.9)	(8.7)
20 krad	2.4	5.6	6.3	8.7	6.8	7.3	10.3
		(8.6)	(9.7)	(11.6)	(5.7)	(6.1)	(8.9)
30 krad	5.7	8.4	8.0	11.8	8.3	6.9	12.8
		(11.9)	(13.0)	(14.9)	(9.0)	(9.4)	(12.2)

* Percentages estimated as the product of the two respective control values

** The sum of frequencies of the two respective controls.

mutagen when applied in a repeated treatment in the successive generation was less than that of the first treatment. The spectrum of chlorophyll mutants in the M_3^2 generation presented in Table XXXIII did not indicate any consistent difference from single treatments either in recurrent or in alternate treatments.

c. Presoaking of seeds

i) M_1 generation

The percentages of survival and seedling height and frequency of chlorophyll deficient chimeras estimated in the M_1 generation following treatment of seeds with EMU after presoaking for different periods are presented in Table XXXIV. The three series corresponding to the three dose-duration combinations differed in the degree of induced lethality and injury at all periods of presoaking. The intensity of effects on these biological criteria was as follows.
2.91 mM for 4hours > 2.91 mM for 2hours > 0.97 mM for 4 hours. A higher dose for a shorter period was thus, more effective in inducing damage in the M_1 generation than a lower dose for a longer period.

In each series the percentages of survival and seedling height decreased steadily with increasing periods of presoaking up to 32 hours. During the 32 to 48 hour period the decrease was not as drastic as that observed in the presoaking period upto 32 hours and the mean values for survival and seedling height had gradually increased towards that of the control. The results, therefore, indicated the existence of a stage

TABLE XXXIII

Relative percentages of different types (spectrum) of chlorophyll mutants in the third generation (M_3^2) in repeated mutagen treatments

Mutagen and dose		Total No.of mutants	Relative percentages of chlorophyll mutants					
First treatment	Second treatment		A	X	V	C	AV	S
1) Recurrent treatment								
Gamma rays	Gamma rays							
..	10 krad	51	33	12	22	31	2	..
..	20 ,,	73	39	..	23	..	37	1
..	30 ,,	59	39	..	49	4	5	3
10 krad	..	16	..	75	25	..	..	..
,,	10 krad	118	68	8	5	3	13	3
,,	20 ,,	71	34	..	37	1	28	..
,,	30 ,,	103	54	10	19	12	5	..
20 krad	..	36	31	..	..	47	22	..
,,	10 krad	25	32	28	20	16	..	4
,,	20 ,,	37	62	..	5	22	..	11
,,	30 ,,	79	42	15	8	33	2	2
30 krad	..	76	20	41	6	24	3	6
,,	10 krad	79	29	3	53	4	..	11
,,	20 ,,	77	42	15	9	34	..	..
,,	30 ,,	100	12	25	43	11	9	..
11) Alternate treatment								
Gamma rays	NMU							
..	0.97 mM	44	43	..	..	..	32	25
..	1.94 ,,	31	16	..	16	7	45	16
..	2.91 ,,	42	48	..	..	31	14	7
10 krad	0.97 ,,	39	61	..	23	13	3	..
,,	1.94 ,,	15	93	..	..	..	..	7
,,	2.91 ,,	25	60	4	20	8	4	4
20 krad	0.97 ,,	89	30	7	21	34	6	2
,,	1.94 ,,	85	28	1	37	33	1	..
,,	2.91 ,,	114	61	..	17	22	..	..
30 krad	0.97 ,,	83	46	8	24	1	18	3
,,	1.94 ,,	89	60	..	12	15	12	1
,,	2.91 ,,	103	20	9	19	45	7	..

TABLE XXXIV

Survival, seedling height and chlorophyll deficient chimeras in the M_1 generation following treatment with NMU after different periods of presoaking

Mutagen dose and duration of treatment	Germination age (period of pre-soaking)	Survival (% of control)		Seedling height (% of control)	No. of plants surviving at maturity	Chlorophyll chimeras	
		30th day	At maturity	30th day		No.	Per 100 M_1 plants
Control	..	100	100	100	227	0	..
0.97 mM for 4 hours	8 hours	100	100	98	227	0	..
	12 ,,	100	99	95	225	1	0.4
	16 ,,	99	99	92	224	1	0.4
	20 ,,	98	100	84	226	0	..
	24 ,,	99	98	84	223	4	1.8
	28 ,,	100	100	77	226	4	1.8
	32 ,,	99	100	76	227	1	0.4
	36 ,,	100	99	78	225	6	2.7
	40 ,,	100	101	78	230	7	3.0
	44 ,,	98	99	78	225	6	2.7
	48 ,,	100	97	79	221	8	3.7
2.91 mM for 2 hours	8 hours	98	97	93	220	0	..
	12 ,,	100	100	90	228	0	..
	16 ,,	100	98	90	222	0	..
	20 ,,	100	94	80	214	1	0.5
	24 ,,	97	92	66	208	3	1.4
	28 ,,	94	81	54	184	4	2.2
	32 ,,	92	74	52	169	7	4.1
	36 ,,	88	78	56	177	7	4.0
	40 ,,	89	83	59	189	5	2.6
	44 ,,	89	82	62	185	1	0.5
	48 ,,	89	91	64	206	4	1.9
2.91 mM for 4 hours	8 hours	100	102	92	232	2	0.9
	12 ,,	98	101	90	230	0	..
	16 ,,	92	93	84	210	6	2.9
	20 ,,	63	60	66	136	6	4.4
	24 ,,	23	18	48	40	0	..
	28 ,,	7	5	33	11	0	..
	32 ,,	5	3	27	7	0	..
	36 ,,	6	5	27	12	0	..
	40 ,,	14	11	29	26	3	11.5
	44 ,,	19	12	33	27	3	11.1
	48 ,,	26	20	39	46	3	6.5

of maximum sensitivity corresponding to the period of presoaking for 32 hours. The peak stage for sensitivity remained the same irrespective of the dose of the mutagen and the criteria adopted for assessing M_1 damage. However, the intensity of damage was more at the higher dose and greater in terms of lethality than injury (Figure 44-i). The frequencies of chlorophyll chimeras were also higher during the intermediate periods of presoaking.

ii) M_2 generation

The frequency of chlorophyll mutations estimated as mutations per 100 M_1 spikes and mutants per 100 M_2 seedlings are presented in Table XXXV. The frequencies increased in both the series with increasing periods of presoaking but the increase was not consistent at all stages (Figure 44-ii). The most conspicuous increase in frequencies was from 16 to 20 hours of presoaking in the lower dose series and from 24 to 28 hours in the higher dose series. The highest frequencies in either of the series were around 40 hours.

The spectrum of chlorophyll mutants presented in Table XXXVI indicated that the relative percentages of the different types of mutants were influenced by the period of presoaking. Albinos were present at all the stages but their relative percentages increased with an increase in the period of presoaking. Viridis mutants were more frequent at short periods of presoaking. Chlorina, alboviridis and striata mutants were induced at all stages but their frequencies did not bear any relationship with the period of presoaking. Xanthas and tigrinas appeared only from the 20 hour period

TABLE XXXV.

Frequency of chlorophyll mutations in the M_2 generation following treatment with EMU after different periods of presoaking

Mutagen/ dose and duration of treatment	Germination age (Period of presoaking)	No.of M_1 plant progenies		No.of M_1 ear progenies		Mutation frequency		% of mutants in mutated ears
		Scored	Segregating	Scored	Segregating	Per 100 M_1 plants	Per 100 M_1 spikes (ears)	
Control	..	25	0	121	0	..	..	..
0.97 mM for 4 hours	8 hours	100	3	445	3	3.0	0.7	6.5
	12 "	100	6	455	7	6.0	1.5	7.4
	16 "	100	9	447	9	9.0	2.0	8.2
	20 "	100	25	410	33	25.0	8.0	11.5
	24 "	100	23	436	37	23.0	8.5	10.9
	28 "	100	13	428	23	13.0	5.4	13.5
	32 "	100	23	402	28	23.0	7.0	11.5
	36 "	100	19	377	29	19.0	7.7	15.6
	40 "	104	24	428	36	23.8	8.4	13.1
	44 "	100	24	348	43	24.0	12.4	17.7
	48 "	101	25	426	38	25.0	8.9	13.5
2.91 mM for 2 hours	8 hours	102	12	394	12	11.8	3.0	12.4
	12 "	98	14	392	19	14.3	4.8	11.6
	16 "	102	18	413	23	17.6	5.6	10.2
	20 "	100	12	415	13	12.0	3.1	8.0
	24 "	101	15	394	25	14.8	6.3	11.7
	28 "	99	25	391	46	25.3	11.7	13.2
	32 "	100	18	437	37	18.0	8.5	10.8
	36 "	97	25	430	58	25.8	13.5	16.8
	40 "	100	32	412	76	32.0	18.4	15.8
	44 "	100	28	379	59	28.0	15.6	16.1
	48 "	100	24	391	51	24.0	13.0	16.2

TABLE XXXVI

Relative percentages of different types (spectrum) of chlorophyll mutants in the M_2 generation following treatment with NMU after different periods of pre-soaking

Mutagen, dose and duration of treatment	Germination age (period of pre-soaking)	Total No. of mutants	Relative percentages of M_2 chlorophyll mutants							
			A	X	V	O	AV	S	T	Others*
0.97 mM for 4 hours	8 hours	8	13	..	87	..	..	..	..	..
	12 "	26	15	..	..	77	..	8	..	..
	16 "	24	17	..	67	8	4	4	..	..
	20 "	145	19	8	21	11	19	6	6	10
	24 "	163	18	16	42	7	4	7	..	6
	28 "	146	26	..	16	42	14	..	..	2
	32 "	149	32	3	42	19	1	..	3	..
	36 "	184	13	8	28	26	9	1	10	5
	40 "	185	35	..	31	21	2	1	5	5
	44 "	346	37	4	21	6	19	..	3	14
	48 "	251	37	1	4	29	10	1	18	..
2.91 mM for 2 hours	8 hours	61	11	..	52	30	5	..	..	2
	12 "	79	11	..	29	5	14	5	..	36
	16 "	107	12	..	17	10	46	2	..	13
	20 "	49	10	..	45	33	..	12	..	..
	24 "	124	15	3	36	13	27	..	6	..
	28 "	283	12	2	37	2	25	..	..	22
	32 "	187	33	..	9	10	8	1	18	21
	36 "	417	30	1	37	17	4	3	..	8
	40 "	707	40	7	18	12	7	..	..	16
	44 "	588	57	5	21	10	6	1	..	..
	48 "	504	30	17	32	3	6	1	..	11

* M and BL

Figure 44. Effect of the length of presoaking on treatment with NMU.

i. Survival and seedling height.

Survival at 30 days.

1) 0.97 mM for 4 hours.

2) 2.91 ,, ,, 2 ,,

3) 2.91 ,, ,, 4 ,,

Seedling height at 30 days

4) 0.97 mM for 4 hours

5) 2.91 ,, ,, 2 ,,

6) 2.91 ,, ,, 4 ,,

ii. Chlorophyll mutation frequency.

Number of mutations per 100 M_1 plants

1) 0.97 mM for 4 hours

2) 2.91 ,, ,, 2 ,,

Number of mutations per 100 M_1 spikes

3) 0.97 mM for 4 hours

4) 2.91 ,, ,, 2 ,,

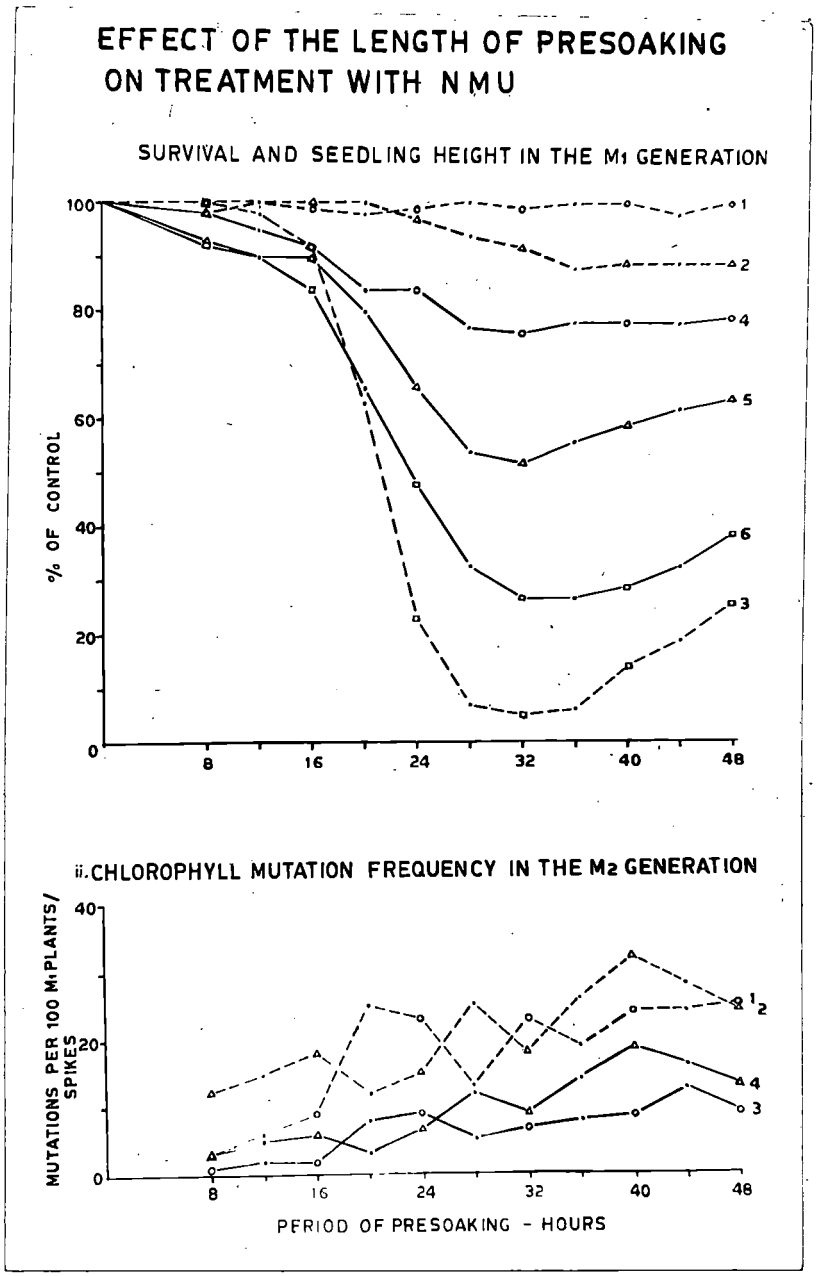

EFFECT OF THE LENGTH OF PRESOAKING ON TREATMENT WITH N M U
SURVIVAL AND SEEDLING HEIGHT IN THE M1 GENERATION
% OF CONTROL
100
80
60
40
20
0
8
16
24
32
40
48
1
2
4
5
6
3
ii. CHLOROPHYLL MUTATION FREQUENCY IN THE M2 GENERATION
MUTATIONS PER 100 M1 PLANTS/ SPIKES
40
20
0
8
16
24
32
40
48
1 2
4
3
PERIOD OF PRESOAKING - HOURS

Figure 44

onwards in both the series and their frequencies were very low. The delayed appearance of xanthas and tigrinas and the increase in the relative percentages of albinas with increasing periods of presoaking were the characteristic features of the chlorophyll mutant spectrum.

More than one type of mutations were observed in certain of the ear-progenies and their estimated frequencies are presented in Table XXXVII. The frequency of multiple mutations was found to increase with an increase in the period of presoaking. The mean number of mutations per mutated ear also increased correspondingly.

The mean segregation ratios presented in Table XXXV indicated that the segregation ratios increased with increasing periods of presoaking. The percentages of ear-progenies with higher percentages of recessive mutants were also found to be more at increased periods of presoaking (Table XXXVIII).

IV. The study of mutated sectors and alteration of mutant frequencies

a. Segregation ratios

One hundred and sixty five M_1 ear-progenies segregating in the M_2 generation for chlorophyll and other mutations with frequencies of mutants significantly lower than 25 per cent were selected for the study of mutated sectors. In each of these progenies, the percentages of M_2 recessive mutants, M_2 heterozygotes and M_3 recessive mutants were estimated.

TABLE XXXVII

Frequency and percentage of M_1 ear progenies segregating for single and multiple chlorophyll mutations following treatment with NMU after different periods of pre-soaking

Mutagen, dose and duration of treatment	Germination age (period of pre-soaking)	No. of ear progenies segregating	Chlorophyll mutations		Ear progenies segregating for mutations of:					
			Total No.	Mean No. per ear	One type	Two types	Three types	One type	Two types	Three types
					Frequency			Relative %		
0.97 mM for 4 hours	8 hours	3	3	1.00	3	..	..	100	..	..
	12 "	7	7	1.00	7	..	..	100	..	..
	16 "	9	9	1.00	9	..	..	100	..	..
	20 "	33	36	1.09	30	3	..	91	9	..
	24 "	37	37	1.00	37	..	..	100	..	..
	28 "	23	24	1.04	22	1	..	96	4	..
	32 "	28	29	1.04	27	1	..	96	4	..
	36 "	29	32	1.10	26	3	..	90	10	..
	40 "	36	37	1.03	35	1	..	97	3	..
	44 "	43	44	1.02	42	1	..	98	2	..
	48 "	38	41	1.08	35	3	..	92	8	..
2.91 mM for 2 hours	8 hours	12	12	1.00	12	..	..	100	..	..
	12 "	19	21	1.11	17	2	..	89	11	..
	16 "	23	23	1.00	23	..	..	100	..	..
	20 "	13	13	1.00	13	..	..	100	..	..
	24 "	25	27	1.08	23	2	..	92	8	..
	28 "	46	47	1.02	45	1	..	98	2	..
	32 "	37	37	1.00	37	..	..	100	..	..
	36 "	58	59	1.02	57	1	..	98	2	..
	40 "	76	90	1.18	63	12	1	83	16	1
	44 "	59	63	1.07	55	4	..	93	7	..
	48 "	51	60	1.18	45	3	3	88	6	6

TABLE XXXVIII

Segregation percentages of chlorophyll mutations in the M_2 generation following treatment with NMU after different periods of pre-soaking

Mutagen, dose and duration of treatment	Germination age (period of pre-soaking)	Total No. of mutations	Relative percentages of M_1 ear progenies showing recessive mutant percentages of:						
			<5	5-10	10-15	15-20	20-25	25-30	>30
0.97 mM for 4 hours	8 hours	3	..	100	..	..	..	..	..
	12 "	7	43	29	14	..	14	..	..
	16 "	9	56	11	11	22	..	..	..
	20 "	36	22	30	25	14	..	3	6
	24 "	37	19	35	5	25	5	8	3
	28 "	24	17	17	21	33	..	12	..
	32 "	29	31	21	21	7	10	10	..
	36 "	32	13	22	19	19	12	9	6
	40 "	37	16	32	19	16	11	3	3
	44 "	44	11	27	11	14	9	7	21
	48 "	41	19	32	10	10	7	12	10
2.91 mM for 2 hours	8 hours	12	17	34	8	8	25	..	8
	12 "	21	19	28	23	10	10	..	10
	16 "	23	26	26	9	22	17	..	..
	20 "	13	39	15	31	15	..	..	..
	24 "	27	22	37	7	11	19	..	4
	28 "	47	13	26	19	17	17	4	4
	32 "	37	22	32	19	13	8	3	3
	36 "	59	14	17	15	15	14	8	17
	40 "	90	21	20	18	17	11	8	5
	44 "	63	16	16	14	24	16	6	8
	48 "	60	12	28	15	23	15	5	2

The observed values for M_2 heterozygotes were calculated as a percentage of the number of plant progenies segregating in the M_3 generation to the total number of progenies scored. The expected percentages were calculated by the formula proposed by Kawai and Sato (1965) i.e., $\frac{(2 \times M_2 \text{ segregation ratio}) \ 100}{100 - M_2 \text{ segregation ratio}}$. The ratios of M_3 mutants were estimated as the mean percentages of all the segregating progenies in the M_3 generation derived from a single M_1 ear.

Based on a comparison of the percentages of M_2 mutants, M_2 heterozygotes and M_3 mutants relating to the same M_1 ear, the progenies were classified into the following five segregation types.

1) This represented progenies with deficit of mutants and a corresponding deficit in the frequency of heterozygotes, as indicated by almost similar observed and expected percentages, in the M_2 generation. M_3 mutant ratios did not differ significantly from 25 per cent indicating a normal monogenic segregation. The data relating to the apical (28 Nos.) and primary axillary (23 Nos.) M_1 ears are presented in Tables XXXIX and XL respectively.

ii) This comprised of progenies with deficit of mutants and a corresponding deficit in the frequency of heterozygotes in the M_2 generation. The M_3 ratios were less than 25 per cent but higher than the M_2 ratios. The data relating to the apical (17 Nos.) and primary axillary (11 Nos.) M_1 ears are presented in Tables XLI and XLII respectively.

iii) Progenies with deficit of mutants and a

corresponding deficit of heterozygotes in the M_2 generation along with a strong deficit in the M_3 were grouped in this category. But in this case the M_2 and M_3 ratios relating to the same M_1 ear were almost similar. Data relating to the 29 M_1 ears are presented in Table XLIII.

iv) Progenies, with deficit of mutants without deficit of heterozygotes, as indicated by the higher observed percentages than the expected ones, in the M_2 generation were recognised under a separate group. The M_3 ratios also showed deficit of recessive mutants and the M_2 and M_3 ratios corresponding to the same M_1 ear were mostly similar. Data relating to 11 M_1 ears are presented in Table XLIV.

v) Progenies with deficit of mutants without deficit of heterozygotes in the M_2 generation but with higher ratios in the M_3 than in the M_2 were grouped under this category. In several progenies the M_3 ratios were lower than 25 per cent. The percentages are given separately for the apical (21 Nos.) and primary axillary (23 Nos.) M_1 ears in Tables XLV and XLVI respectively.

b. Size of the mutated sectors

The data relating to segregation types (i) and (ii) above were utilised for the estimation of the size of the mutated sector of the M_1 ear. Only about one half of the 165 ear-progenies studied, i.e., 53 of type (i) and 28 of type (ii), were suitable for this estimation. The remaining progenies were unsuitable due to various types of abnormal segregations. In type (i), since the percentages of mutants and heterozygotes in the M_2 generation were significantly and proportionately

less than normal and the M_3 mutant percentages did not deviate significantly from 25 per cent, the deficit of M_2 mutants could be attributed to the chimeric constitution of the M_1 ear. The magnitude of reduction in the M_2 ratio as compared to the M_3 ratio of the corresponding progeny was therefore, proportional to the size of the mutated sector. In type (ii) the reduction in percentages of M_3 mutants could be attributed to the elimination of mutant types at the haplontic stage or to the interaction of genes governing the mutant phenotype. However, since the percentages of mutants and heterozygotes in the M_2 generation were proportional and the M_2 ratios were significantly lower than the corresponding M_3 ratios, the deviations in M_2 from the M_3 were taken as due to the formation of sector in the M_1 ear. As such, these data were also utilised for the estimation of the size of the mutated sector.

The size of the mutated sector of the M_1 ear was estimated by dividing the percentage of mutants in the M_2 generation in each progeny by that in the corresponding M_3 progeny. The sector sizes so calculated for the different M_1 ears are presented separately for the apical ear (Tables XXXIX and XLI) and primary axillary ear (Tables XL and XLII). The sector size showed considerable variation from ear to ear. In each ear category the sectors following chemical mutagen treatments especially EMS were smaller than those following radiation treatments. The sectors on the primary axillary ear were comparatively larger than those on the apical ear.

TABLE XXXIX

Estimation of the size of the mutated sector of the apical ear
— Progenies with normal segregation ratios in the M_3 generation

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants				Mutated sector size M_2/M_3	No. of initial cells M_3/M_2
	No. of M_2 plants	Mutants No.	%	Progenies of M_2 plants Scored	Segregating	% of heterozygotes Observed	Expected	No. of M_3 plants	Mutants No.	%	χ^2* (3:1)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
i) Gamma rays													
Albina	47	1	2.1	42	3	7	4	224	51	22.7	0.06	0.09	11
,,	27	2	7.4	25	5	20	16	319	69	21.6	2.02	0.34	3
,,	69	8	11.6	59	10	17	26	653	173	26.4	0.81	0.44	2
Nonviable	52	3	5.8	10	1	10	12	233	58	24.9	0.01	0.23	4
ii) Fast neutrons													
Albina	60	2	3.3	40	4	10	7	472	101	21.4	3.27	0.15	7
Xantha	33	2	6.1	30	6	20	13	419	105	25.0	0.01	0.24	4
,,	39	1	2.6	31	3	10	6	258	60	23.2	0.33	0.11	9
Chlorina	15	1	6.6	12	1	8	14	99	23	23.2	0.21	0.28	4
,,	96	2	2.1	63	1	2	4	61	14	22.9	0.09	0.09	11
Nonviable	33	3	9.1	29	9	31	20	214	44	20.6	2.24	0.44	2

* No value is significant at P = 0.05

TABLE XXXIX (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
iii) EMS													
Albina	139	13	9.4	40	12	30	21	1356	308	22.7	3.77	0.41	2
,,	90	8	8.9	39	4	10	19	420	88	20.9	3.67	0.43	2
,,	141	2	1.4	127	1	1	3	80	16	20.0	1.07	0.07	14
,,	100	2	2.0	85	1	1	4	152	43	28.2	0.88	0.07	14
,,	76	1	1.3	60	1	2	3	37	8	21.6	0.24	0.06	16
,,	87	4	4.6	82	13	16	10	1626	376	23.1	2.96	0.20	5
,,	99	2	2.0	85	1	1	4	54	12	22.2	0.24	0.09	11
Xantha	90	3	3.3	39	4	10	7	365	80	21.9	1.77	0.15	7
,,	90	3	3.3	83	6	7	7	965	241	25.0	0.01	0.13	8
Chlorina	35	1	2.9	29	1	3	5	129	30	23.3	0.16	0.12	8
,,	101	3	3.0	95	12	13	6	1907	448	23.5	2.35	0.13	8
iv) EMU													
Albina	84	2	2.4	73	5	7	5	608	156	25.6	0.14	0.09	11
Striata	94	2	2.2	43	1	2	4	76	18	23.6	0.07	0.09	11
Stunted	64	2	3.1	20	1	5	6	218	52	23.9	0.15	0.13	8
Nonviable	41	3	7.3	10	2	20	16	345	77	22.3	1.25	0.32	3
Dwarf	81	6	7.4	10	2	20	16	309	77	25.0	0.01	0.30	3
v) DES													
Albina	80	6	7.5	40	7	18	16	603	136	22.5	1.99	0.33	3
Striata	50	1	2.0	30	1	3	4	127	28	22.0	0.67	0.09	11

TABLE XL

Estimation of the size of the mutated sector of the primary axillary ear

— Progenies with normal segregation ratios in the M_3 generation

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants				Mutated sector size M_2/M_3	No. of initial cells M_3/M_2
				Progenies of M_2 plants		% of heterozygotes							
	No. of M_2 plants	Mutants		Scored	Segregating	Observed	Expected	No. of M_3 plants	Mutants				
		No.	%						No.	%	χ^2* (3:1)		
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
i) Gamma rays													
Albina	78	5	6.4	24	3	12	14	799	189	23.6	0.81	0.27	4
,,	58	4	6.9	54	14	26	15	1002	239	23.8	0.64	0.29	3
ii) Fast neutrons													
Albovirids	65	2	3.1	52	3	6	6	453	117	25.8	0.19	0.12	8
Defective	27	1	3.7	25	2	8	8	234	58	24.8	0.01	0.15	7
iii) EMS													
Albina	48	2	4.2	31	3	10	9	658	162	24.6	0.05	0.17	6
,,	46	3	6.5	29	3	10	14	543	136	25.0	0.01	0.26	4

* No value is significant at P = 0.05

TABLE XL (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
Albina	82	5	6.1	61	6	10	13	442	99	22.3	1.59	0.27	4
"	74	2	2.7	64	1	2	6	129	28	21.7	0.66	0.12	8
"	56	2	3.6	45	7	16	8	1222	287	23.5	1.49	0.15	7
"	47	2	4.2	42	1	2	9	136	34	25.0	0.00	0.17	6
"	75	2	2.7	64	1	2	6	125	33	26.4	0.13	0.10	10
"	47	2	4.3	41	5	12	9	498	121	24.2	0.13	0.18	6
Xantha	56	3	5.4	45	1	2	11	154	31	20.1	1.92	0.27	4
"	66	2	3.0	59	2	3	6	287	73	25.4	0.03	0.12	8
"	57	3	5.3	52	6	12	11	907	208	22.9	2.12	0.23	4
"	49	3	6.1	29	4	14	13	495	108	21.8	2.75	0.28	4
Chlorina	73	3	4.1	44	3	7	9	446	106	23.7	0.36	0.17	6
Albovirids	75	2	2.7	65	5	8	6	255	55	21.5	1.68	0.13	8
"	47	2	4.3	38	5	13	9	325	73	22.4	1.05	0.19	5
Tigrina	74	2	2.7	64	1	2	6	139	28	20.1	1.87	0.13	7
"													
iv) <u>NMU</u>													
Xantha	103	13	12.6	37	10	27	20	1088	252	23.2	1.96	0.54	2
Chlorina	98	2	2.0	75	6	8	4	771	170	22.0	3.65	0.09	11
Sterile	97	6	6.2	20	2	10	13	222	62	27.9	1.00	0.22	5
v) <u>DES</u>													
Albina	42	4	9.5	24	2	8	21	260	55	21.1	2.05	0.45	2
"	50	4	8.0	28	7	25	17	1357	312	23.0	2.87	0.35	3

TABLE XLI

Estimation of the size of the mutated sector of the apical ear — Progenies with deficit of recessive mutants in the M_2 and M_3 generations

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants			Mutated sector size M_2/M_3	No. of initial cells M_3/M_2
	No. of M_2 plants	Mutants No.	%	Progenies of M_2 plants Scored	Segregating	% of heterozygotes Observed	Expected	No. of M_3 plants	Mutants No.	%		
i) Gamma rays												
Albina	45	1	2.2	44	4	9	5	76	12	15.7	0.14	7
ii) Fast neutrons												
Albina	39	1	2.6	29	2	7	5	98	9	9.1	0.29	4
"	96	2	2.1	63	1	2	4	83	13	15.6	0.13	7
Alboviridis	42	1	2.4	39	3	8	5	182	17	9.3	0.26	4
"	52	1	1.9	44	2	5	4	297	56	18.8	0.10	10
iii) EMS												
Albina	38	2	5.3	26	4	15	11	133	23	17.2	0.31	3
"	30	1	3.3	20	3	15	7	443	69	15.6	0.21	5
"	58	1	1.7	41	3	7	3	318	40	12.5	0.14	7
Xantha	53	4	7.5	36	7	19	16	798	151	18.9	0.40	3
Striata	39	1	2.6	30	1	3	5	50	9	18.0	0.14	7
Alboviridis	45	1	2.2	31	3	10	5	430	85	19.7	0.11	9
"	39	3	7.7	30	2	7	17	103	18	17.4	0.44	2
Minute	76	1	1.3	66	2	3	3	168	15	8.3	0.16	6
Nonviable	98	1	1.0	65	5	6	2	643	48	7.5	0.13	8
iv) DES												
Xantha	39	1	2.6	31	3	10	5	299	51	17.0	0.15	7
v) MMS												
Albina	121	11	9.1	23	3	13	20	68	12	17.6	0.52	2
Chlorina	57	1	1.8	12	1	8	4	20	2	10.0	0.18	6

TABLE XLII

Estimation of the size of the mutated sector of the primary axillary ear — Progenies with deficit of recessive mutants in the M_2 and M_3 generations

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants			Mutated sector size M_2/M_3	No. of initial cells M_3/M_2
	No. of M_2 plants	Mutants No.	Mutants %	Progenies of M_2 plants Scored	Progenies of M_2 plants Segregating	% of heterozygotes Observed	% of heterozygotes Expected	No. of M_3 plants	Mutants No.	Mutants %		
i) Gamma rays												
Albina	42	1	2.4	29	1	3	5	62	11	17.7	0.14	7
Chlorina	18	1	5.6	15	1	7	12	91	17	18.6	0.30	3
Albovirids	69	1	1.4	36	1	3	3	175	7	4.0	0.35	3
ii) Fast neutrons												
Albina	56	2	3.6	37	4	11	8	215	33	15.3	0.24	4
iii) EMS												
Albina	44	1	2.3	29	2	7	5	138	10	7.2	0.32	3
"	47	1	2.1	41	3	7	4	308	58	18.8	0.11	9
"	25	1	4.0	17	1	6	8	81	13	16.0	0.25	4
Albovirids	38	1	2.6	21	1	5	5	165	19	11.5	0.23	4
Nonviable	79	3	3.8	67	3	4	8	273	19	7.0	0.54	2
Lazy	44	1	2.3	43	1	2	5	124	15	12.1	0.19	5
iv) MNU												
Albina	80	4	5.0	30	5	17	11	173	34	19.6	0.26	4

c. Number of initial cells

The size of the mutated sector is inversely proportional to the number of initial cells contributing to the formation of the generative tissue of the ear, i.e., the smaller the sector the larger the number of initial cells. The number of functional initial cells relating to each ear is calculated by dividing the percentage of M_3 mutants by that of M_2 mutants of the concerned progeny, corrected to the nearest integers and the estimates are presented in Tables XXXIX to XLII. In each ear category the estimated number of initial cells was found to be larger, following treatment with chemical mutagens than with radiations. For the apical ear the maximum number of effective initial cells was 11 with gamma rays, fast neutrons and NMU and 16 with EMS. For the primary axillary ear the maximum numbers were seven with gamma rays, eight with fast neutrons, 10 with EMS and 11 with NMU. Thus, the numbers were larger for the apical ear (11 and 16) than for the primary axillary ear (7 to 11).

d. Deficit of recessive mutants

Segregation types (iii), (iv) and (v) described above represented deficit of recessive mutants of various categories. A comparative study of Tables XLIII and XLIV revealed that haplontic elimination was more frequent (29 progenies) than diplontic elimination (11 progenies). In the segregation type (iii), represented by ear-progenies in Table XLIII, the almost equal reduction in M_2 and M_3 mutant percentages and a proportionate reduction in M_2 heterozygotes indicated that the deficit was due to an elimination of cells carrying

TABLE XLIII

Percentages of mutants and heterozygotes in the M_2 and M_3 generations in ear progenies with deficit of recessives and M_2 heterozygotes

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants		
	No. of M_2 plants	Mutants		Progenies of M_2 plants		% of heterozygotes		No. of M_3 plants	Mutants	
		No.	%	Scored	Segregating	Observed	Expected		No.	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
i) Gamma rays										
Albina	49	7	14.3	21	8	38	33	821	82	10.0
"	26	1	3.8	21	1	5	8	230	13	5.6
"	35	1	2.9	21	1	5	6	64	3	4.5
Chlorina	15	3	20.0	10	4	40	50	506	91	18.0
Alboviridis	33	6	18.2	26	12	46	44	1762	355	20.1
"	28	5	17.9	21	8	38	44	933	160	17.1
ii) Fast neutrons										
Albina	52	1	1.9	40	4	10	4	400	15	3.8
"	40	5	12.5	17	3	18	28	133	16	12.0
Chlorina	25	4	16.0	18	6	33	38	390	54	13.8
Alboviridis	24	3	12.5	9	4	44	28	401	63	15.7
Lethal dwarf	24	5	20.8	19	9	47	52	153	23	15.0
Nonviable	31	5	16.1	24	2	8	38	136	24	17.6
"	29	4	13.8	23	11	47	32	412	85	20.6

TABLE XLIII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
iii) EMS										
Albina	53	10	18.9	39	11	28	42	741	105	14.1
"	39	4	10.2	30	1	3	25	55	7	12.7
"	17	3	18.2	13	2	15	44	200	25	12.5
"	32	4	12.5	15	1	7	28	149	23	15.4
Xantha	50	6	12.0	28	10	36	27	967	64	6.6
Alboviridis	37	1	2.7	18	1	6	6	91	4	4.3
Stunted	74	16	21.6	20	8	40	55	2501	434	17.4
Nonviable	43	7	16.3	30	8	27	39	179	27	15.1
Ageotropic	30	5	16.7	20	4	20	40	688	126	18.3
Open spikelet	90	14	15.6	50	11	22	37	1085	232	21.4
iv) NMU										
Xantha	46	9	19.6	10	1	10	49	131	17	12.9
Alboviridis	56	10	17.9	10	5	50	44	1201	196	16.3
Stunted	56	9	16.1	20	9	45	38	1645	290	17.6
v) DMS										
Viridis	43	8	18.6	24	7	29	46	1324	239	18.0
vi) NMS										
Dwarf	126	13	10.3	27	4	15	23	139	24	17.3
"	60	6	10.0	17	3	18	22	1118	15	10.1

TABLE XLIV

Percentages of mutants and heterozygotes in the M_2 and M_3 generations in ear progenies with deficit of recessives but without deficit of M_2 heterozygotes

Mutagen and type of mutant	M_2 mutants			M_2 heterozygores				M_3 mutants		
				Progenies of M_2 plants		% of heterozygotes				
	No. of M_2 plants	Mutants		Scored	Segregating	Observed	Expected	No. of M_3 plants	Mutants	
		No.	%						No.	%
1) Gamma rays										
Albina	30	5	16.7	7	6	86	40	481	101	20.9
Xantha	17	2	11.8	14	9	64	27	772	151	19.5
Chlorina	38	4	10.5	18	14	78	23	2750	457	16.4
"	30	4	13.3	17	11	65	31	2221	414	18.6
ii) Fast neutrons										
Stunted	49	8	16.3	20	14	70	39	732	171	23.4
Nonviable	46	5	10.9	25	14	56	24	275	47	17.1
iii) EMS										
Chlorina	37	7	18.9	18	13	72	47	389	93	23.9
Tigrina	6	1	16.7	5	4	80	40	188	19	10.1
iv) NMU										
Alboviridis	51	7	13.7	21	15	71	32	2368	463	19.5
"	79	13	16.5	10	6	60	40	969	159	16.4
Nonviable	97	14	14.4	20	10	50	34	2445	540	22.0

mutations at the haploid phase. The segregation type (iv) represented by ear-progenies in Table XLIV gave a different picture. The reduction in M_2 and M_3 mutant percentages without a reduction of M_2 heterozygotes indicated an elimination of mutants at the diplontic stage due to lethality of the zygote or proembryo carrying the recessive mutant gene in the homozygous condition.

The segregation type (v) represented by ear-progenies in Tables XLV and XLVI indicated a more complex type of deficit of recessive mutants. The difference in the proportion of M_2 mutants and heterozygotes indicated elimination of mutants at the diplontic stage. The percentages of M_3 mutants in many progenies were significantly lower than 25. This deficit could be attributed to either haplontic elimination of mutants or complex gene interactions controlling the mutant phenotype. Moreover, the M_3 mutant percentages were significantly higher than the M_2 percentages in every progeny indicating the chimeric condition of the M_1 ear. Thus, the segregation pattern of this type could be due to a combination of two or more of the several phenomena leading to the deficit of recessive mutants such as haplontic and diplontic elimination of mutant genotypes, chimeric nature of M_1 ears and complex interactions of genes governing the mutant characters. This forms a major category represented by 44 ear-progenies out of the 165 investigated.

e. Excess of recessive mutants

In 37 M_1 ear-progenies the percentages of recessive

TABLE XLV

Percentages of mutants and heterozygotes in the M_2 and M_3 generations in apical ear progenies with deficit of M_2 mutants and with or without deficit of M_2 heterozygotes and M_3 mutants

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants		
				Progenies of M_2 plants		% of heterozygotes				
	No. of M_2 plants	Mutants		Scored	Segregating	Observed	Expected	No. of M_3 plants	Mutants	
		No.	%						No.	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
i) Gamma rays										
Albina	11	1	9.1	10	7	70	20	1731	335	19.3
"	29	1	3.4	28	6	21	7	509	66	12.9
Alboviridis	35	1	2.9	31	19	61	6	3458	700	23.1
Minute	48	5	10.4	20	7	35	23	1372	316	23.0
Semidwarf	75	6	8.0	10	3	30	17	388	102	26.3
ii) Fast neutrons										
Albina	68	2	2.9	40	10	25	6	751	176	23.4
Xantha	53	2	3.8	36	12	33	8	694	146	21.0
Chlorina	68	5	7.4	40	15	38	16	1119	208	18.5
Tall lethal	28	1	3.6	16	4	25	7	280	66	23.5
Winged	40	3	7.5	35	10	29	16	759	192	25.3

TABLE XLV (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
iii) EMS										
Albina	49	4	8.2	30	10	33	18	1712	426	24.8
"	53	3	5.7	36	13	36	12	1037	225	21.6
"	37	2	5.4	30	9	30	11	869	120	13.4
Striata	105	2	1.9	79	11	14	4	1762	205	11.6
Alboviridis	38	1	2.6	26	5	19	5	147	26	17.6
Minata	46	2	2.2	44	14	32	4	811	93	11.5
Semidwarf	42	5	11.9	20	8	40	27	525	112	21.3
Mottled gluze	83	7	8.4	25	9	36	18	1160	258	22.2
iv) NHU										
Striata	31	1	3.2	21	7	33	7	721	159	22.0
v) DES										
Albina	43	1	2.3	24	8	33	5	1755	365	20.7
Alboviridis	56	1	1.8	16	2	12	4	325	74	22.7

TABLE XLVI

Percentages of mutants and heterozygotes in the M_2 and M_3 generations in primary axillary ear progenies with deficit of M_2 mutants and with or without deficit of M_2 heterozygotes and M_3 mutants

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes				M_3 mutants		
				Progenies of M_2 plants		% of heterozygotes				
	No. of M_2 plants	Mutants		Scored	Segregating	Observed	Expected	No. of M_3 plants	mutants	
		No.	%						No.	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
i) Gamma rays										
Albina	34	1	2.9	27	5	18	6	671	173	25.8
Xantha	46	2	4.3	10	2	20	9	183	50	27.3
Viridis	26	2	7.7	24	11	46	17	1907	369	19.3
Chlorina	22	1	4.5	21	8	38	9	705	137	19.4
ii) Fast neutrons										
Viridis	26	2	7.7	14	6	43	17	673	84	12.4
iii) EMS										
Albina	73	9	12.3	44	23	52	28	820	207	25.2
"	65	2	3.1	59	8	14	6	1242	296	23.8
"	110	3	2.7	97	15	15	5	2709	663	24.4
"	30	3	10.0	15	7	46	22	771	139	18.0

TABLE XLVI (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
Xantha	35	1	2.9	17	6	35	6	321	24	7.4
Viridis	30	2	6.7	15	6	40	14	739	157	21.2
Chlorina	32	1	3.1	23	12	52	6	592	72	12.1
"	29	1	3.4	25	9	36	7	382	63	16.4
Alboviridis	44	2	4.5	29	13	45	9	428	75	17.5
Minute	29	1	3.4	25	9	36	7	66	7	10.6
Nonviable	37	3	8.1	21	9	43	18	1160	240	20.7
Dwarf	11	1	9.1	8	3	38	20	431	117	27.1
Semidwarf	76	9	11.8	25	12	48	27	1898	511	26.9
iv) NMU										
Albina	24	1	4.2	18	4	22	9	452	93	20.5
Xantha	48	2	4.2	20	8	40	9	1149	260	22.6
Tigrina	92	5	5.4	79	20	25	11	1126	277	24.6
v) DES										
Striata	48	4	8.3	30	14	47	18	2862	507	17.7
vi) NMS										
Chlorina	68	8	11.8	17	10	59	27	404	105	25.9

mutants in the M_2 generation were more than the expected maximum of 25. These progenies were carried forward to the M_3 generation on M_2 plant progeny basis and the percentages of M_2 heterozygotes and M_3 mutants were estimated. The data are presented in Table XLVII. The percentages of M_2 mutants ranged from 28.4 to 80.0 but in 19 out of 37 progenies the increase was not significant. This apparent increase in the percentages of M_2 mutants might be due to the small size of the M_1 ear-progenies resulting from the very high M_1 sterility. Significant increase in percentages was more frequent in combination treatments and treatment with chemical mutagens. The frequency of heterozygotes also showed a corresponding increase in most of the progenies.

The percentages of M_3 mutants, however, did not always correspond to that in the M_2. In 24 out of 37 progenies the M_3 percentages did not differ significantly from 25. Eleven progenies showed recessive deficit and the remaining two yielded a significant excess of recessive mutants. Several progenies with recessive deficit in the M_3 generation were derived from M_2 progenies segregating normally. The two cases of recessive excess were represented by a viridis mutant induced by EMS and a dwarf macro-mutant induced by NMU. The mean percentages of these recessive mutants were 27.6 and 44.5 respectively.

V. Micro-mutations

The mean, standard error and variance in respect of the five quantitative characters in the M_2, M_3 and M_4 generations following treatment with gamma rays and EMS

TABLE XLVII

Segregation ratios of ear progenies with excess of mutants in the M_2 generation

Mutagen and type of mutant	M_2 mutants			M_2 heterozygotes			M_3 mutants			
	No. of M_2 plants	Mutants		Progenies of M_2 plants		% of heterozygotes	No. of M_3 plants	Mutants		
		No.	%	Scored	Segregating			No.	%	χ^2 (3:1)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
i) Gamma rays										
Albina	26	8	30.8	7	5	71	306	61	20.0	4.18*
"	2	1	50.0	1	1	100	299	75	25.0	0.01
"	36	14	38.9	21	14	67	808	184	22.7	2.13
"	14	6	42.9	7	4	57	346	80	23.1	0.64
Chlorina	27	9	33.3	17	10	59	1117	254	22.7	2.98
Alboviridis	37	12	32.4	23	16	70	2471	522	21.1	19.88*
Striata	36	12	33.3	20	16	80	1217	287	23.6	1.27
Winged	18	8	44.4	10	4	40	205	37	18.0	5.11*
"	25	8	32.0	10	4	40	262	55	21.0	2.24
ii) X-rays										
Albina	71	29	40.8**	32	25	78	2116	503	23.8	1.71
Viridis	96	33	34.4**	58	44	76	3712	876	23.6	5.88*

* Deficit of mutants
** Excess of mutants
} Significant at $P = 0.05$

TABLE XLVII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
iii) Fast neutrons										
Albina	67	25	37.3**	41	26	64	744	205	27.5	2.59
Viridis	75	25	33.3	46	31	67	2867	753	26.2	2.41
Narrow leaved	18	7	38.9	10	5	50	581	136	23.4	0.75
Reduced lemma	39	13	33.3	25	11	44	982	211	21.5	6.47*
iv) EMS										
Xantha	49	17	34.7	30	23	77	3475	831	23.9	2.21
Viridis	65	23	35.4**	35	26	74	2112	516	24.4	0.36
"	58	26	44.8**	30	25	83	2431	672	27.6	8.99**
"	30	12	40.0	16	14	88	971	251	25.2	0.35
"	73	35	47.9**	38	32	84	2318	575	24.8	0.04
"	62	27	43.5**	31	24	77	2069	468	22.6	6.19*
Chlorina	28	12	42.9**	8	4	50	291	87	29.8	3.58
Tigrina	36	12	33.3	22	18	82	1570	337	21.5	10.45*
v) MMU										
Albina	64	25	39.1**	38	27	71	1892	471	24.9	0.01
"	35	15	42.9**	10	8	80	1068	202	18.9	21.09*
Xantha	78	25	32.1	49	35	71	2678	567	21.2	20.91*
Viridis	46	16	34.8	10	7	70	1682	434	25.8	0.57
Macro-mutant	109	31	28.4	36	27	75	5066	2254	44.5	V.high**

TABLE XLVII (CONTD.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
vi) EMS										
Chlorina	69	24	34.8	36	26	72	2709	615	22.6	7.58*
,,	69	35	50.7**	31	29	94	2216	512	23.1	4.24*
vii) Fast neutrons + NMU										
Albina	31	13	41.9**	16	11	69	868	221	25.5	0.09
Chlorina	44	29	65.9**	12	11	92	2029	474	23.4	2.87
,,	20	16	80.0**	3	3	100	509	121	23.8	0.37
,,	48	29	60.4**	16	13	81	1998	496	24.8	0.04
,,	32	18	56.2**	11	10	91	1477	387	26.2	1.17
,,	38	26	68.4**	11	10	91	1777	429	24.2	0.68
,,	60	36	60.0**	20	16	80	1813	461	25.4	0.19

were estimated. These estimates were made dose-wise in each mutagen by combining the data on all the 50 progenies in each dose.

a. Duration

The mean duration expressed as days to flowering in Table XLVIII did not differ significantly in any of the doses and generations. On the other hand, the variance increased with increasing doses in each mutagen, the magnitude of the increase being more after treatment with gamma rays than with EMS. The increase in variance though recorded in all the three generations was most conspicuous in the M_4 generation. The distribution of frequencies (Figure 45—i) was almost normal in all generations.

b. Height of plants

The mean height of plants was not influenced by any of the doses of gamma rays or EMS. (Table XLIX). Variance was found to increase with increasing doses of the mutagens, the magnitude of increase being considerably more following treatment with gamma rays than with EMS. The maximum increase in variance was recorded in the M_3 generation where the variance at 30 krad of gamma rays was more than double that of the untreated population. From M_3 to M_4 generation the variance at all dose levels decreased. The increase in variance was symmetrical as could be seen in Figure 45(ii).

c. Number of ears

The mean number of ears per plant in each of the doses

TABLE XLVIII

Mean, standard error and variance in the M_2 to M_4 generations for duration — Days to flowering

Mutagen and dose	No. of plants studied	Range of variation (Days)	Mean (Days)	Mean as % of control	S.E. of the mean	Variance	Variance as % of control
M2 generation							
Control	500	81-93	87.6	100	0.08	3.43	100
Gamma rays							
10 krad	500	84-96	88.4	101	0.08	3.39	99
20 "	500	82-96	88.5	101	0.08	3.60	105
30 "	500	83-96	88.6	101	0.10	4.52	132
EMS - 38 mM	500	84-95	89.0	102	0.08	3.57	104
77 "	500	84-95	89.1	102	0.08	3.46	101
115 "	500	85-96	89.9	103	0.09	3.88	113
M3 generation							
Control	1200	85-102	93.8	100	0.09	10.28	100
Gamma rays							
10 krad	1200	85-106	94.3	101	0.09	10.99	106
20 "	1200	84-107	93.9	100	0.11	13.95	126
30 "	1200	83-107	92.8	99	0.11	13.51	124
EMS - 38 mM	1200	83-105	92.6	99	0.09	10.36	101
77 "	1200	83-104	92.2	98	0.09	10.90	106
115 "	1200	82-105	93.3	99	0.10	11.67	112
M4 generation							
Control	1200	82-102	87.7	100	0.08	8.49	100
Gamma rays							
10 krad	1200	82-107	88.3	101	0.10	12.67	149
20 "	1200	82-107	88.2	101	0.10	12.37	146
30 "	1200	81-106	87.8	100	0.10	11.76	138
EMS - 38 mM	1200	81-101	87.7	100	0.09	10.84	128
77 "	1200	82-103	87.1	99	0.09	9.49	112
115 "	1200	82-105	88.0	100	0.10	11.17	132

TABLE XLIX

Mean, standard error and variance in the M_2 to M_4 generations for plant height

Mutagen and dose	No. of plants studied	Range of variation (cm)	Mean (cm)	Mean as % of control	S.E. of the mean	Variance	Variance as % of control
M_2 generation							
Control	500	96-130	113.0	100	0.28	37.4	100
Gamma rays							
10 krad	500	94-132	112.9	100	0.28	37.9	101
20 ,,	500	95-132	112.1	99	0.34	56.8	152
30 ,,	500	80-128	108.4	96	0.40	78.3	209
EMS - 38 mM	500	90-128	111.8	99	0.28	38.3	102
77 ,,	500	93-129	111.9	99	0.28	40.1	107
115 ,,	500	95-132	114.1	101	0.30	45.4	121
M_3 generation							
Control	1200	76-116	98.7	100	0.17	35.0	100
Gamma rays							
10 krad	1200	70-116	97.9	99	0.20	47.2	135
20 ,,	1200	69-116	98.2	99	0.21	55.1	157
30 ,,	1200	63-117	96.5	98	0.25	72.8	208
EMS - 38 mM	1200	75-117	99.3	101	0.17	35.3	101
77 ,,	1200	70-118	99.5	101	0.17	36.4	104
115 ,,	1200	70-118	99.6	101	0.19	44.8	128
M_4 generation							
Control	1200	84-119	99.9	100	0.18	40.6	100
Gamma rays							
10 krad	1200	77-119	99.0	99	0.20	46.9	115
20 ,,	1200	75-120	98.2	98	0.19	43.9	108
30 ,,	1200	73-119	97.4	97	0.22	56.6	139
EMS - 38 mM	1200	79-121	99.9	100	0.19	43.7	108
77 ,,	1200	75-121	99.5	100	0.20	47.3	116
115 ,,	1200	78-125	100.5	101	0.20	48.0	118

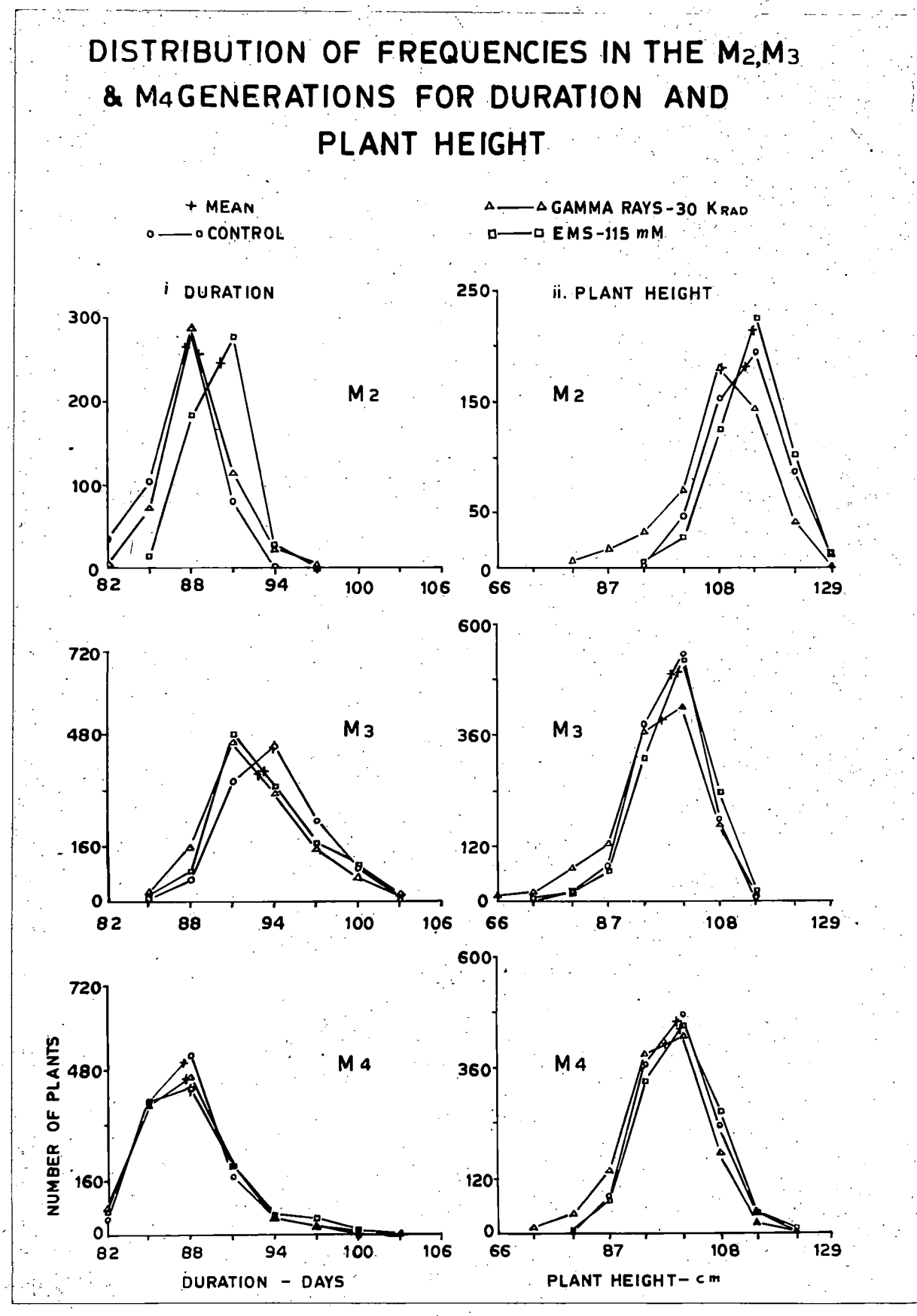

DISTRIBUTION OF FREQUENCIES IN THE M2,M3 & M4 GENERATIONS FOR DURATION AND PLANT HEIGHT
+ MEAN
o——o CONTROL
Δ——Δ GAMMA RAYS-30 KRAD
□——□ EMS-115 mM
i DURATION
ii. PLANT HEIGHT
M2
M3
M4
NUMBER OF PLANTS
DURATION - DAYS
PLANT HEIGHT- cm

Figure 45

indicated that there was slight increase in means at the higher doses of gamma rays and EMS in the M_2 generation, but as generations advanced the means gradually reverted to the control value (Table I). The M_4 means did not show any difference from the mean of the control. Variance significantly increased and the increase was proportional to the dose following treatment with either of the mutagens. The magnitude of increase was considerably more in treatment with gamma rays than with EMS. The maximum variance following treatment with gamma rays was in the M_2 generation, whereas the highest values with EMS treatment were obtained in the M_3 generation. With advancing generations, the variance gradually decreased and approached the control value. The increase in variance, however, was not symmetrical, the values towards large number of ears being frequent. This made the distribution of frequencies skewed on the positive side as seen in Figure 46(i).

d. Length of ear

The mean length of ear did not differ significantly in any of the doses and generations (Table II). On the other hand, the variance increased considerably and the increase was progressive with increasing doses of the mutagens. The magnitude of increase was more in the case of gamma rays than with EMS. The maximum variance in both gamma rays and EMS was recorded in the M_2 generation. With advancing generations the degree of increase in variance was low. The distribution of frequencies was normal as indicated in Figure 46(ii).

TABLE L

Mean, standard error and variance in the M_2 to M_4 generations for number of ears per plant

Mutagen and dose	No. of plants studied	Range of variation	Mean (No.)	Mean as % of control	S.E. of the mean	Variance	Variance as % of control
M2 generation							
Control	500	2-13	5.8	100	0.09	4.11	100
Gamma rays							
10 krad	500	2-14	5.8	100	0.09	4.16	101
20 ,,	500	2-16	6.7	115	0.11	6.05	147
30 ,,	500	2-16	6.4	110	0.11	6.39	155
EMS - 38 mM	500	2-15	6.1	105	0.09	4.08	99
77 ,,	500	2-15	6.3	109	0.09	4.38	107
115 ,,	500	2-15	6.2	107	0.10	4.63	113
M3 generation							
Control	1200	2-11	5.2	100	0.05	2.79	100
Gamma rays							
10 krad	1200	1-13	5.2	100	0.05	3.07	110
20 ,,	1200	1-13	5.4	104	0.05	3.56	128
30 ,,	1200	1-14	5.4	104	0.05	3.43	123
EMS - 38 mM	1200	1-13	5.5	105	0.05	3.28	118
77 ,,	1200	1-13	5.4	104	0.05	3.14	113
115 ,,	1200	1-14	5.7	109	0.05	3.48	125
M4 generation							
Control	1200	2-13	5.3	100	0.05	2.61	100
Gamma rays							
10 krad	1200	1-12	5.2	98	0.05	2.99	115
20 ,,	1200	1-12	5.1	96	0.05	2.91	111
30 ,,	1200	2-13	5.3	100	0.05	3.07	118
EMS - 38 mM	1200	2-12	5.4	102	0.05	2.74	105
77 ,,	1200	1-11	5.2	98	0.05	2.63	101
115 ,,	1200	1-12	5.3	100	0.05	2.73	105

TABLE LI

Mean, standard error and variance in the M_2 to M_4 generations for length of ear — Main ear

Mutagen and dose	No. of plants studied	Range of variation (mm)	Mean (mm)	Mean as % of control	S.E. of the mean	Variance	Variance as % of control
M_2 generation							
Control	500	150-250	209	100	0.64	208	100
Gamma rays							
10 krad	500	145-250	208	100	0.77	298	143
20 "	500	155-260	214	102	0.74	272	131
30 "	500	145-255	210	100	0.86	368	177
EMS - 38 mM	500	170-250	209	100	0.62	192	92
77 "	500	155-255	210	100	0.71	255	122
115 "	500	160-250	210	100	0.75	281	135
M_3 generation							
Control	1200	150-245	196	100	0.47	268	100
Gamma rays							
10 krad	1200	145-245	193	98	0.48	276	103
20 "	1200	140-245	194	99	0.51	308	115
30 "	1200	135-245	193	98	0.53	332	124
EMS - 38 mM	1200	140-245	196	100	0.47	267	100
77 "	1200	135-250	196	100	0.46	260	97
115 "	1200	140-250	195	100	0.50	302	113
M_4 generation							
Control	1200	145-245	201	100	0.44	229	100
Gamma rays							
10 krad	1200	145-245	198	99	0.47	270	118
20 "	1200	135-255	202	100	0.47	262	114
30 "	1200	140-250	199	99	0.50	296	129
EMS - 38 mM	1200	140-245	201	100	0.45	248	108
77 "	1200	145-245	201	100	0.46	258	112
115 "	1200	145-240	200	100	0.44	233	102

DISTRIBUTION OF FREQUENCIES IN THE M_2, M_3 & M_4 GENERATIONS FOR NUMBER AND LENGTH OF EARS

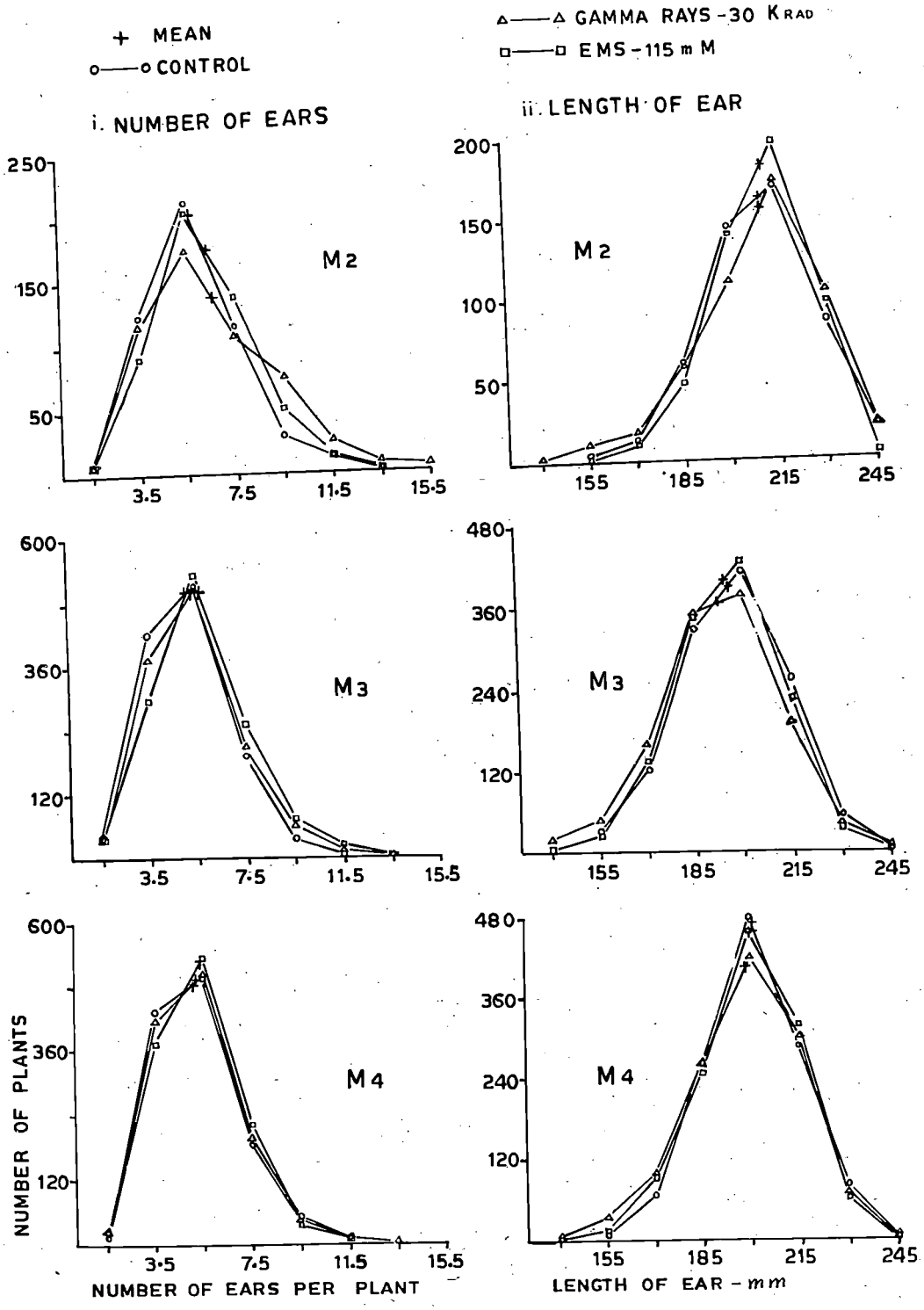


Figure 46

e. Number of spikelets

The mean number of spikelets per panicle did not differ significantly from that of the control in any of the doses and generations (Table LII). Variance, however, increased and the increase was more conspicuous after treatment with gamma rays especially, at the highest dose (30 krad). Maximum variance was recorded in the M_2 generation. The magnitude of increase in variance was less with advancing generations and almost approached the control value in the M_4 generation. The distribution of progeny means was normal as indicated in Figure 47. The wider spread in the M_2 generation was because of the smaller number of means used in plotting the distribution.

In general, the mutagens at the doses tried did not significantly alter the means of populations with respect to the characters studied except for the small increase in the number of ears per plant. On the other hand, the mutagens were highly effective in increasing variability for all the characters. The increase in variance was progressive with an increase in the dose of the mutagens. The magnitude of the increase was always larger with gamma rays than with EMS irrespective of the character and generation, thereby indicating that at the level of the doses employed, gamma rays were more effective in inducing micro-mutations than EMS. The magnitude of variability induced was found to differ with the character. With the same dose of gamma rays, the highest induced variability was for height of plants and the characters

TABLE LII

Mean, standard error and variance in the M_2 to M_4 generations for number of spikelets per panicle — Main panicle

Mutagen and dose	No. of plants studied	Range of variation	Mean (No.)	Mean as % of control	S.E. of the mean	Variance	Variance as % of control
M2 generation							
Control	50	73-114	95.1	100	1.41	99	100
Gamma rays							
10 krad	50	74-116	99.5	105	1.40	97	98
20 "	50	76-119	96.0	101	1.44	104	105
30 "	50	73-121	98.7	104	1.58	124	125
EMS - 38 mM	50	80-118	96.7	102	1.41	99	100
77 "	50	71-114	93.1	98	1.50	112	113
115 "	50	73-114	95.6	101	1.44	103	104
M3 generation							
Control	150	58-93	77.6	100	0.60	54	100
Gamma rays							
10 krad	150	57-101	75.8	98	0.61	55	102
20 "	150	59-99	75.6	97	0.59	52	96
30 "	150	53-97	76.5	99	0.67	67	124
EMS - 38 mM	150	59-93	76.2	98	0.59	53	98
77 "	150	61-97	75.9	98	0.58	50	93
115 "	150	63-95	77.4	100	0.58	51	94
M4 generation							
Control	150	66-98	82.9	100	0.59	53	100
Gamma rays							
10 krad	150	62-102	82.3	99	0.60	54	102
20 "	150	69-106	84.3	102	0.62	58	109
30 "	150	63-104	82.9	100	0.63	60	113
EMS - 38 mM	150	68-103	83.6	101	0.63	60	113
77 "	150	66-109	85.0	103	0.63	60	113
115 "	150	62-107	83.2	100	0.61	55	104

DISTRIBUTION OF PROGENY MEANS IN THE M_2, M_3 & M_4 GENERATIONS FOR NUMBER OF SPIKELETS

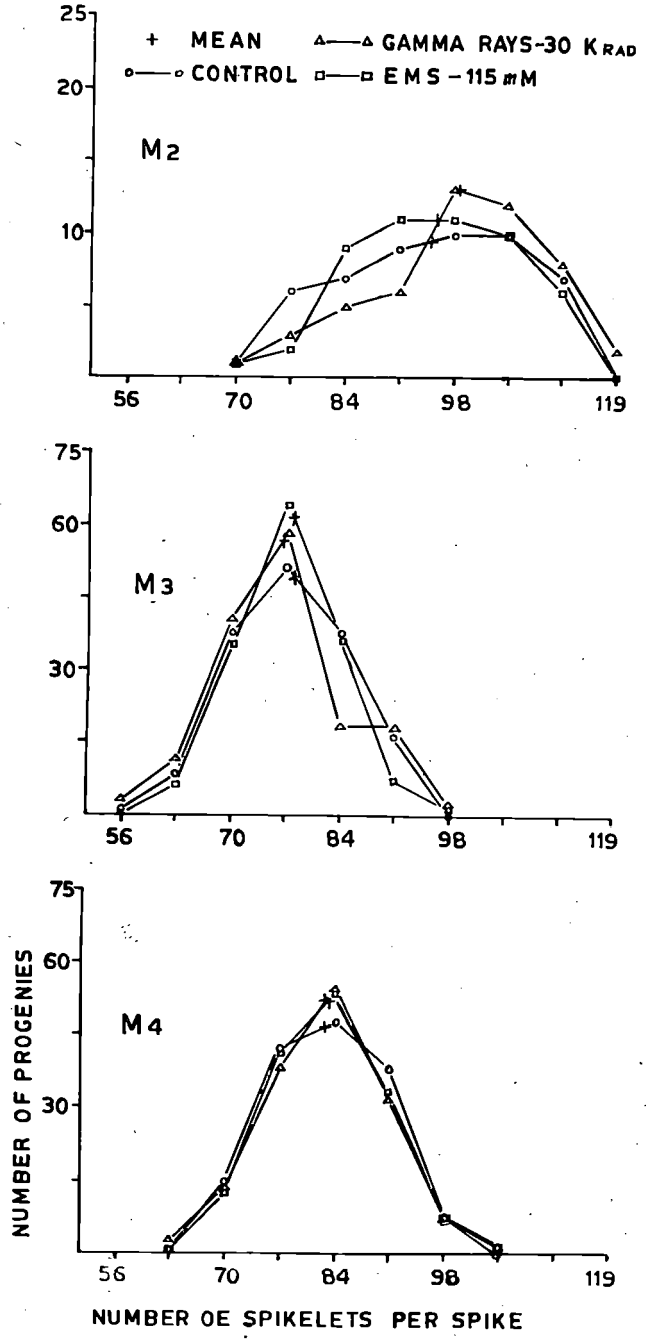


Figure 47

arranged in order of decreasing variance were length of ear, number of ears, duration and number of spikelets. Therefore, micro-mutations affecting height of plants might have been most frequently induced. For the same character the variability was found to differ between generations. Maximum variability was recorded for the number of ears, length of ear and number of spikelets in the M_2 generation, for height of plants in the M_2 and M_3 and for duration in the M_4 generation. The increase in variance was symmetrical in all characters except the number of ears per plant. The symmetrical increase in variability might indicate the incidence of micro-mutations in the positive and negative directions with almost equal frequencies.

VI. Radiosensitivity of varieties

a. M_1 generation

The effect of X-rays at six dose levels in the range of 5 to 20 krad on survival, seedling height and seed fertility of 20 varieties of rice was studied in the M_1 generation. The highest dose was found to be lower than the LD_{50} for the different criteria of M_1 damage such as lethality, injury and sterility and therefore, a comparative study of radiosensitivity of these varieties was made on the basis of effects induced by the highest dose (20 krad). The percentages of survival, seedling height and seed fertility for the 20 varieties are presented in Table LIII. The results indicated that the different types of M_1 damages were induced in different intensities

TABLE LIII

Effect of X-rays on survival, seedling height and seed fertility in the M_1 generation of different varieties

Dose - 20 krad

Varieties	% of control			
	Survival 30th day	Seedling height		Seed fertility
		15th day	30th day	
INDICAS				
1. Co.29	96	82	93	48.8
2. Co.10	98	84	96	68.0
3. Co.13	91	80	90	68.1
4. Co.18	98	80	93	60.8
5. Adt.27	94	82	87	56.1
6. Adt.28	94	79	93	47.8
7. Tkm.6	96	73	89	52.2
8. Ptb.10	97	67	79	53.4
9. Ptb.23	96	76	87	60.7
10. Ptb.31	98	76	84	59.4
11. Peta	96	76	79	50.1
JAPONICAS				
12. Norin-1	95	67	87	67.3
13. Norin-6	96	74	88	67.3
14. Norin-17	95	69	84	73.5
15. Rikku-132	97	79	82	55.8
OTHERS				
16. Taichung native-1	98	78	84	68.2
17. I.R.8	95	83	88	70.9
18. Tainan-3	89	71	81	76.8
19. Taichung-65	84	65	84	73.9
20. Kaohsiung-68	85	75	89	75.6

by the same dose of radiation. In the varieties studied, the ranges were 2 to 16 per cent for lethality, 4 to 21 per cent for injury and 23 to 52 per cent for sterility indicating that the intensity of the induced damage increased in the order lethality, injury and sterility.

b. M_2 generation

The frequency of chlorophyll mutations was estimated as the number of mutants per 100 M_2 seedlings at the doses, 15 and 20 krad. The results are presented in Table LIV. In most of the varieties the frequency of chlorophyll mutants increased with an increase in dose from 15 to 20 krad. The frequencies for the 20 varieties differed considerably with a range of 0.42 to 2.47 at 20 krad. The relative percentages of chlorophyll mutants were estimated by combining the frequencies of mutants at the doses 15 and 20 krad and presented in Table LV. Albina and viridis mutants were induced in all varieties, whereas chlorina and alboviridis were recovered from most of them. Xantha and striata mutants were induced in about half the number of varieties and tigrina was present only in two varieties.

c. Measures of radiosensitivity

The varieties studied were found to show differential sensitivity to X-rays irrespective of whether the criterion for comparison was a measure of M_1 damage such as lethality,

TABLE LIV

Frequency of chlorophyll mutations in the M_2 generation following X-ray irradiation of different varieties

Varieties	15 krad		20 krad		Mutant frequency (per 100 M_2 seedlings)	
	No. of M_2		No. of M_2			
	Seedlings	Mutants	Seedlings	Mutants	15 krad	20 krad
INDICAS						
1. Co.29	4977	87	5053	95	1.75	1.88
2. Co.10	3147	52	2943	47	1.65	1.60
3. Co.13	1824	18	1688	7	0.99	0.42
4. Co.18	2796	13	2186	36	0.46	1.65
5. Adt.27	4721	35	5018	31	0.74	0.62
6. Adt.28	3213	61	2776	57	1.90	2.05
7. Tkm.6	2581	67	2269	56	2.21	2.47
8. Ptb.10	2858	58	2192	48	2.03	2.19
9. Ptb.25	3647	65	3047	45	1.78	1.48
10. Ptb.31	2522	53	2120	43	2.10	2.03
11. Peta	2977	14	2139	35	0.47	1.63
JAPONICAS						
12. Norin-1	3004	20	2513	22	0.67	0.88
13. Norin-6	2756	35	2851	60	1.27	2.10
14. Norin-17	2542	16	2513	11	0.63	0.44
15. Rikku-132	2587	36	1614	23	1.39	1.43
OTHERS						
16. Taichung Native-1	3119	75	3158	58	2.40	1.84
17. I.R.8	4430	79	3606	52	1.78	1.44
18. Tainan-3	6127	38	4579	39	0.62	0.85
19. Taichung-65	5000	39	3800	20	0.78	0.50
20. Kaohsiung-68	6784	84	5120	86	1.24	1.68

TABLE IV

Relative percentages of different types (spectrum) of chlorophyll mutants in the M_2 generation following X-ray irradiation of different varieties (Doses 15 and 20 krad combined)

Varieties	No.of mutants	Relative percentages						
		A	X	V	C	AV	B	T
INDICAS								
1. Co.29	182	39	1	27	10	15	7	1
2. Co.10	99	51	29	15	4	..	1	..
3. Co.13	25	52	12	20	16	..	..	..
4. Co.18	49	74	..	20	4	2	..	..
5. Adt.27	66	21	..	11	50	18	..	..
6. Adt.28	118	48	..	31	10	9	2	..
7. Tkm.6	123	57	..	11	19	13	..	..
8. Ptb.10	106	44	..	41	..	..	1	14
9. Ptb.23	110	52	2	30	3	12	1	..
10. Ptb.31	96	56	23	13	1	7	..	..
11. Peta	49	76	2	22	..	..	..	..
JAPONICAS								
12. Norin-1	42	41	2	21	36	..	..	..
13. Norin-6	95	41	..	19	8	20	12	..
14. Norin-17	27	59	19	11	..	11	..	..
15. Rikku-132	59	60	..	22	8	2	8	..
OTHERS								
16. Taichung Native-1	133	49	..	50	..	1	..	..
17. I.R.8	131	44	..	11	38	7	..	..
18. Tainan-3	77	23	3	18	51	4	1	..
19. Taichung-65	64	10	..	12	11	64	3	..
20. Kaohsiung-68	170	37	..	37	14	11	1	..

injury and sterility or an estimate of M_2 chlorophyll mutation frequency (Table LVI). The variation was very high for M_1 sterility ranging from 23 to 52 per cent. Sensitivity differences were of a lower magnitude in respect of lethality and injury. Thus, the degree of sensitivity differences between varieties depended to a certain extent on the criterion adopted to measure the radiation effects. This is illustrated in Figure 48. In Table LVI the varieties belonging to the two subspecies, *indica* and *japonica* were ranked in the order of increasing M_1 damage and M_2 mutant frequencies. It was evident that when different criteria were adopted for measuring radiosensitivity the ranking of varieties in terms of induced damage did not exactly correspond to each other.

TABLE LVI

Varieties ranked (top to bottom) in order of increasing M_1 damage and M_2 mutant frequency
Dose - 20 krad

M_1 lethality (survival reduction at 30 days)		M_1 injury (seedling height reduction at 30 days)		M_1 sterility (seed fertility reduction)		M_2 mutant frequency (Mutants per 100 M_2 plants)	
%	Sl.No.	%	Sl.No.	%	Sl.No.	%	Sl.No.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
2	2*	4	2*	23.2	18	0.42	3*
2	4*	7	1*	24.4	20	0.44	14
2	10*	7	4*	26.1	19	0.50	19
2	16	7	6*	26.5	14	0.62	5*
3	8*	10	3*	29.1	17	0.85	18
3	15	11	7*	31.8	16	0.88	12
4	1*	11	20	31.9	3*	1.43	15
4	7*	12	13	32.0	2*	1.44	17
4	9*	12	17	32.7	12	1.48	9*
4	11*	13	5*	32.7	13	1.60	2*
4	13	13	9*	39.2	4*	1.63	11*
5	12	15	12	39.3	9*	1.65	4*
5	14	16	10*	40.6	10*	1.68	20
5	17	16	14	43.9	5*	1.84	16
6	5*	16	16	44.2	15	1.88	1*
6	6*	16	19	46.6	8*	2.03	10*
9	3*	18	15	47.8	7*	2.05	6*
11	18	19	18	49.9	11*	2.10	13
15	20	21	8*	51.2	1*	2.19	8*
16	19	21	11*	52.2	6*	2.47	7*

The numbers in columns 2, 4, 6 & 8 are serial numbers of varieties as given in Table LV.

* indica varieties

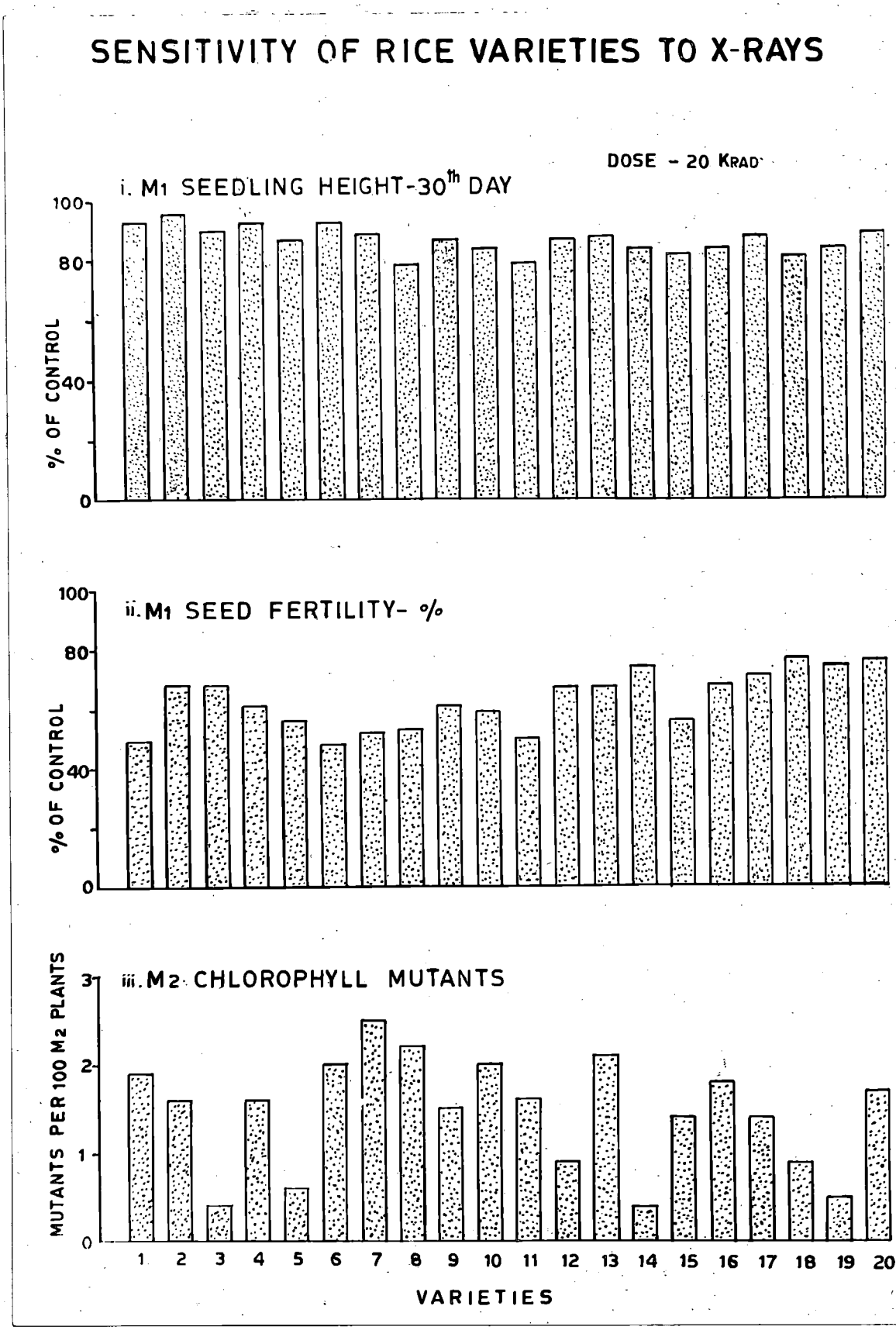

SENSITIVITY OF RICE VARIETIES TO X-RAYS
DOSE - 20 KRAD
i. M1 SEEDLING HEIGHT-30th DAY
% OF CONTROL
100
80
40
0
ii. M1 SEED FERTILITY- %
% OF CONTROL
100
80
40
0
iii. M2 CHLOROPHYLL MUTANTS
MUTANTS PER 100 M2 PLANTS
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
VARIETIES

Figure 48

DISCUSSION

DISCUSSION

The success of mutation breeding largely depends on our understanding of the process of induction, repair and recovery of mutations. Basic information on the type and doses of mutagens, the frequency and spectrum of mutations induced, the relative effectiveness and efficiency of different mutagens and the correlation of M_1 effects with M_2 mutations is essential in utilising mutation breeding effectively for rice improvement. Problems related to the enhancement of mutagenic efficiency, recovery of induced mutations, induction of micro-mutations and differential sensitivity of varieties are particularly important in this context. The present study was undertaken to investigate these aspects which would enhance the possibilities for inducing and recovering viable mutants of practical value. The results obtained are discussed in the following sections in the light of information already available.

I. Effects of mutagens in the M_1 generation

Physical and chemical mutagens were reported to induce the following three types of effects in the biological material (Gaul, 1970).

1) Physiological damage (primary injury),

2) Factor mutations (gene mutations),

and 3) Chromosome mutations (chromosomal aberrations).

For a given mutagenic treatment there is a correlation between M_1 damage and M_2 mutation frequency (Gaul, 1959). Therefore,

a quantitative determination of M_1 damage should be a routine procedure in mutation breeding experiments. Damage to plants in the M_1 generation resulting from the biological effects induced in seeds by mutagens could be measured by several criteria such as,

1) Germination and survival reduction (lethality),
2) Plant growth reduction (injury),
3) Fertility reduction (sterility),
4) Increase in the frequency of chromosomal aberrations, and
5) Increase in the frequency of chlorophyll deficient chimeras.

Germination and survival

Germination of rice seeds was not found to be affected in the present investigation by the radiations (X-rays, gamma rays and fast neutrons) even at very high doses (Table III). The highest dose of 60 krad of gamma rays which induced total seedling lethality did not affect germinability of seeds. But emergence and survival under field conditions were reduced progressively with increasing doses of gamma rays and X-rays. This agreed with the reports of Gaul (1964a, 1970) and Mikaelsen (1968b) in barley. Cytological and anatomical examination of germinating barley seeds by Mikaelsen (1968b) had shown that the initial germination was mainly through cell elongation and this stage was apparently not inhibited by radiation. A reduction in survival even at the advanced stages of growth was evident in the present case indicating a

prolonged lethal action of radiations as reported in rice by Yamagata et al. (1965) and Goud et al. (1967).

In contrast to ionising radiations, chemical mutagens (DES, EMS, MMS, NMU and MNNG) reduced germination and the reduction was progressive with increasing doses. The reduction in germination and survival following treatment with NMU, MNNG and MMS was significant even at very low doses. Among the chemical mutagens, EMS induced the least lethality. Survival reduction due to lethality of seedlings occured in the early seedling stage following treatment with NMU and MNNG. Several reports indicated that chemical mutagens such as DES (Rao and Ayengar, 1964), EMS (Yamagata et al., 1965; Ganeshan, 1970) and NMU (Siddiq and Swaminathan, 1968a) reduced germination of rice seeds considerably. In chemical mutagen treatments in the present investigation, M_1 seedlings reaching the fourth leaf stage (15 days) had almost survived to maturity. D'Amato et al. (1962) reported similar results in wheat. These observations indicate that the nature of lethal effects differs between radiations and chemical mutagens. Radiation-induced lethality was manifest subsequent to germination through a prolonged lethal action even at the advanced stages of plant growth, whereas chemically induced lethality was mostly expressed through inhibition of germination. The highly toxic chemicals such as NMU and MNNG reduced not only germination but also survival during the early seedling stage.

Plant growth

A greater inhibitory effect of radiations and of most

of the chemical mutagens on the root in comparison with the effect on the shoot in germinating seeds was observed in the present experiments. With high doses of gamma rays and NMU the inhibitory effect on the root was twice as intense as that on the shoot (Table IV). However, in treatments with MNNG, the growth of shoot was more affected than that of the root. Kawai (1963a), Simon (1963) and Nyttensere et al. (1965) reported greater inhibitory effect of radiations on the root than on the shoot in rice. Nikaelsen (1968b) and Nikaelsen and Brunner (1968) in barley and Scarascia et al. (1961) in wheat reported that neutron irradiation caused a greater depressing effect on the root than on the shoot.

Radiations as well as chemical mutagens were found in the present investigation, to be effective in reducing the height of plants at the different stages of growth. The magnitude of reduction in height increased progressively with increasing doses of mutagens. Gamma rays and NMU were more effective than the other mutagens in reducing the height of plants. Following irradiation with fast neutrons the plants showed less variability in height than after treatment with X-rays, gamma rays and chemical mutagens. This might indicate a more uniform effect of fast neutrons on the biological material resulting from their relative insensitivity to the action of modifying factors and absence of secondary physiological effects. Similar observations were made by Matsuo et al. (1958), Masima and Kawai (1959) and Yamaguchi (1964) in rice and Caldecott et al.

(1952, 1954), Ehrenberg and Nybom (1954), Rana and Swaminathan (1967) and Goul (1970) in other plants.

Reduction in plant height estimated during the seedling stage was found to be more drastic than at the later stage of growth at each of the doses in every mutagen, thereby indicating an apparent recovery of M_1 plants from injury (Figure 4). The rate of recovery was more at the higher doses. This could be due to the growth of uninjured meristematic cells which replaced the injured ones as growth proceeded.

Several explanations have been offered to account for growth inhibition following mutagen treatment of seeds. Factors such as auxin destruction (Skoog, 1935; Smith and Kersten, 1942), inhibition of auxin synthesis (Gurdon, 1954), failure of assimilatory mechanism (Quastler and Baer, 1950; Riley, 1953), production of diffusible growth retarding substances (MacKey, 1951), changes in the specific activity of enzymes (Haskins and Chapman, 1956; Cherry et al., 1961, 1962; Endo, 1967), delay in the onset of first mitosis (Natarajan, 1958) and inhibition of DNA synthesis (Mikaelsen, 1968b) have so far been attributed from time to time for this growth inhibition.

Fertility

Seed fertility of M_1 plants was found to decrease with increasing doses of radiations (Table V). The decrease in fertility was more drastic upto the middle doses. At high doses the decrease was not proportional to the increase in

dose as may be seen in Figure 3. Similar observations indicating an apparent saturation effect at the higher doses have been made by Ekendahl (1961), Henderson (1963) and Yeh and Henderson (1963) in rice. Fast neutrons per unit dose had reduced fertility more drastically than X-rays and gamma rays. Low fertility following neutron irradiation was reported by Simon (1963), Yeh and Henderson (1963), Yamaguchi (1964) and Siddiq (1967) in rice, MacKey (1952), and Caldecott et al. (1954) in barley, and MacKey (1954) in wheat.

The low incidence of sterility with the use of chemical mutagens was a conspicuous feature observed in the present study. The percentages of fertility estimated at LD_{20} for injury by chemical mutagens were 66.1, 86.8, 96.0 and 98.8 for EMS, MMS, NMU and MNNG respectively, in contrast to 19.0, 35.8 and 44.3 for radiations such as gamma rays, X-rays and fast neutrons respectively. Rao and Ayengar (1964) and Siddiq and Swaminathan (1968a) had also pointed attention to such a feature in rice. Among the chemical mutagens, EMS induced the highest sterility of 33.9 per cent (fertility - 66.1 %) as against 4.0 per cent (fertility - 96.0%) by NMU at LD_{20} for injury. Gavin et al. (1968) found on the contrary, that NMU induced more sterility than EMS in barley.

Radiation-induced sterility might be due to detectable chromosome aberrations and cryptic deficiencies, whereas sterility induced by EMS and other chemical mutagens might be due to cryptic deficiencies and specific gene mutations (Gaul et al.,1966; Bender and Gaul, 1966, 1967; Sato and

Gaul, 1967). Sterility induced by radiations is reported to be mostly haplontic, but a large part of EMS-induced sterility is diplontic in nature (Gaul et al., 1966; Sato and Gaul, 1967). Ekberg (1969) based on an extensive analysis of mutagen-induced sterility in the M_1 generation also came to similar conclusions. In the present investigation, no attempts were made for studying the meiotic aberrations in order to assess the nature and degree of the chromosomal aberrations that were induced. As pointed out by Gaul (1970), sterility counts are better than meiotic investigations for a quantitative determination of the sum total of chromosome mutations which have survived the sporophytic generation.

Relative biological effectiveness

There is conclusive evidence to show that differences exist in the RBE of different radiations. Bora (1961) stated that the effectiveness of radiations increased in the order, gamma rays, X-rays and fast neutrons. However, the present experiments had demonstrated that there was very little difference in the effects of X-rays and gamma rays as reflected in almost similar RBE values obtained for these radiations; fast neutrons were effectively superior to X- and gamma rays. Fujii (1962) and Nilan (1964) reported that the RBE of X-rays and gamma rays were approximately the same. Several reports had repeatedly confirmed that neutrons produced a higher yield of biological events per unit of absorbed energy and so were several times more

effective than sparsely ionising radiations in higher plants (Nender, 1970). In the present study also, the RBE values of fast neutrons were higher in relation to X-rays and gamma rays irrespective of the criteria employed for comparison. For instance, the estimated values based on germination, length of primary root, survival, seedling height and fertility were <29, 8.0, <19, 18.6 and 10.6 respectively as may be seen in Table XX. The values for different criteria of damage in the M_1 generation, thus, ranged from 8 to 29. Higher RBE values for fast neutrons in rice were also reported by Matsumura (1964), Siddiq (1967), Mikaelsen and Navaratna (1968) and Mikaelsen et al. (1968). Differences in RBE values of neutrons for different effects induced by radiations have been reported by Ehrenberg and Nybom (1954) in barley, by Matsumura (1961, 1966) and Matsumura and Nezu (1961) in wheat and by Smith (1969) and Smith et al. (1968) in maize. The RBE values were reported to range from 3 to 20 for different species of wheat (Matsumura and Nezu, 1961), 10 to 15 for diploid wheat (Matsumura, 1966) and 10 to 33 for maize (Smith, 1969; Smith et al., 1968). Thus, the present results clearly demonstrate that in rice fast neutrons are more effective in inducing biological effects than sparsely ionizing X-rays and gamma rays.

Chimeras

Chlorophyll deficient sectors on plants in the M_1 generation were occasionally seen in irradiated material. In the present study, plants with chlorotic streaks appeared

in treatment with fast neutrons at a very low frequency, but not in treatments with X-rays or gamma rays. In contrast to radiations, high frequencies of chlorophyll chimeras were found in treatments with NMU and EMS (Table VI). The frequency of plants with chlorophyll deficient sectors was found to increase progressively with increasing doses of chemical mutagens. At equimolar concentrations NMU was found to induce a higher frequency of chlorophyll chimeras than EMS indicating that NMU was more effective than EMS in this respect. However, at doses producing other comparable biological effects, say LD_{20} for lethality and injury, these chemicals induced almost similar frequencies of chlorophyll chimeras indicating that they were almost equal in efficiency. Chlorophyll deficient sectors on M_1 plants were reported by Shastry and Ramiah (1961), Horvat (1961) and Siddiq (1967) following irradiation in rice. Scarascia et al. (1961) in durum wheat, Fujii (1965) in diploid wheat and Smith et al. (1964) in maize reported that the frequency of chlorophyll chimeras was more following irradiation with fast neutrons than with X-rays. The superiority of chemical mutagens over radiations in inducing chlorophyll deficient sectors was emphasised by several investigators in a number of plant species. The ability of EMS to induce a high frequency of chlorophyll chimeras in the M_1 generation was first reported by Heslot et al. (1959) and later by Favret (1960), Heslot (1961), Heslot et al. (1961), Ramanna and Natarajan (1965), Nageraja Rao and Natarajan (1965) and Natarajan and Shivasankar (1965) in barley. Similar effects were realised in bread wheat by Goud (1965, 1967a),

Swaminathan (1966c), Varughese and Swaminathan (1968) and Prabhakara Rao and Washington (1970) and in durum wheat by Scarascia et al. (1961) and D'Amato et al. (1962). An increased effectiveness of NMU over EMS in inducing chlorophyll chimeras was reported in barley by Swaminathan et al. (1968) and Savin et al. (1968) and in wheat by Prasad (1968). Eriksson and Lindgren (1970) stated that the dose dependence of sector frequency had made possible the use of this criterion as an estimate of mutagenic effects in the M_1 generation.

The variation in the number, size and distribution of chlorophyll deficient sectors observed in the present study was significant. In chimeric plants induced by fast neutrons the chlorophyll deficient sectors were restricted to the first few leaves represented by leaf meristems in the embryo. Whereas, in those induced by NMU and EMS the sectors in most cases persisted to the last leaf; in a few cases they extended even to the panicle. The sectors varied from narrow streaks to very broad ones. They were found to differ in their colour ranging from light green (viridis) to extreme lack of pigmentation (albina). The sectors induced by fast neutrons were predominantly white. Whereas, they were yellow or yellow green following treatment with NMU and EMS. The narrow white sectors induced by fast neutrons and the broad ones with slight colour variation following treatment with NMU and EMS might indicate that the former represented a more

drastic effect than the latter leading to rapid cell elimination as growth proceeded.

Chlorophyll deficient sectors were reported to arise as a result of chromosomal aberrations (Kaplan, 1954; Zacharias and Ehrenberg, 1962; Sybenga, 1964; Fujii, 1965), recessive mutations (Favret, 1960; Gichner and Veleminsky, 1965), dominant mutations (Shama Rao and Sears, 1964; Fujii and Matsumura, 1966), plastid mutations (Hsieh, 1959; Goud, 1967a; Robbelen, 1962) and destruction of chloroplast structure or DNA (Varughese, 1966; Klienhofs, 1969).

Ear chimeras were observed in the present investigation with a very low frequency and consisted of fertile sectors with small grains and awned large grains (Figure 6). These deviating sectors occupied longitudinal contiguous regions on the ear. But they did not breed true in the succeeding generation and therefore, might represent somatic mutations of the nature of periclinal chimeras with the mutation remaining confined to the L_I layer. Chimeras for ear characters were observed in the M_1 generation by Bhaskaran and Swaminathan (1961), Bhatia and Swaminathan (1963), Swaminathan (1966c), Varughese (1966), Varughese and Swaminathan (1968), Rana and Swaminathan (1967), Goud (1967a) and Prasad (1968) in bread wheat, Desai (1969) in durum wheat and Ramanna and Natarajan (1965) in barley following treatment with mutagens. Prasad (1968) observed that the morphological changes were not heritable. On the other hand, Goud (1967a) found that many of the ear chimeras

bred true, thereby indicating that they represented genic or chromosomal changes.

II. Mutations in the M_2 generation

The terminology for characterising the different types of mutations in literature is not uniform. Several terms have been often used without being clearly defined as pointed out by Gaul (1964a). Vital mutations were classified by Gaul (1961b), based on the method of detection that is, whether the mutation could be recognised in a single plant or in a group of plants, into meco-mutations and micro-mutations. Macro--mutations were further classified into transspecific and intraspecific, whereas micro-mutations were grouped into manifest and cryptic. Swaminathan (1964) classified mutations into the following four groups.

i) Micro-mutations: This comprised mutations which could be isolated only through the adoption of biometrical procedures.

ii) Visible mutations: This group included mutations which could be identified either by the naked eye or by the use of appropriate screening procedures. They might be either lethal or viable.

iii) Macro-mutations: Mutations in which the change though inherited as a single unit of recombination, yet affecting a constellation of characters, were grouped under this category.

iv) Systematic mutations: Mutations which either simulated an already existing taxon or necessitated the

creation of a new systematic unit by virtue of the character affected being a key one were included in this group.
In the present investigation, mutations were broadly grouped into chlorophyll and viable for purposes of scoring frequencies and estimating mutagenic effectiveness and efficiency. The chlorophyll mutations were further classified according to the system suggested by Gustafsson (1940) and expanded by Konzak et al. (1968), whereas, the viable mutations were classified on the basis of the scheme proposed by Swaminathan (1964) into macro-mutations, visible mutations and systematic mutations. Micro-mutations, were not taken into consideration for estimation of mutation frequencies, as they could be detected only by the enhanced variability of quantitative traits in advanced generations.

Frequency of mutations

There are three different estimates of mutation frequencies.

1) Number of mutations per 100 M_1 plants (Gustafsson, 1937),

2) Number of mutations per 100 M_1 spikes (Stadler, 1928a), and

3) Number of mutants per 100 M_2 plants (Gaul, 1960).

Here the terms "mutation" and "mutant" mean the mutation event and the plant that phenotypically expresses the mutation respectively. An accurate method of estimating induced mutation frequencies must compensate for the bias introduced by factors such as diplontic selection, small

progeny size and increased size of mutated sector at the higher doses (Milan, 1964). In tillering cereals like rice, barley and wheat where the embryo has already differentiated into a number of spike primordia, method (1) will always make an over-estimation of the mutation frequency in comparison with the other methods. In contrast to method (3), method (2) will be influenced by the size of the mutated sector of the M_1 spike and the progeny size (Gaul, 1960). These may distort the functional relationship between dose and mutational response to a very serious degree. Therefore, the estimate of M_2 mutant frequency will be the best estimate of the initial mutation frequency. According to Konzak et al. (1965a) the M_2 seedling basis for estimating mutation frequencies permits the resolution of separate mutational events occurring within an M_1 spike. Muller (1965) in Arabidopsis also found that the M_2 mutant frequency was the best estimate of mutation frequency especially at higher doses. However, Sarvella et al. (1962) reported that in barley the mutation rates of apical spikes remained independent of the degree of tillering and therefore, a reliable estimate of mutation frequency could be made by utilising the apical spikes for scoring mutations.

Yamaguchi (1963) traced the primary branch of an M_1 ear back to the single cell of the embryo and estimated the mutation frequency per original treated cell as the proportion of primary branches which segregated for mutations. Yamaguchi and Minh (1964) claimed this as the most accurate

method of estimating mutation frequency in irradiated rice seeds. Kawai and Sato (1965) presented a method of calculated mutation frequency per 100 initial cells of M_1 spikes, by dividing the number of mutants per 100 M_2 plants with the estimated number of initial cells per M_1 spike. This estimate was reported to take care of chimerism in M_1 spikes. Hansel (1968) also presented a method for estimating the rate of chlorophyll mutations per 100 M_1 nuclei in barley. However, these methods outlined by Yamaguchi (1963), Kawai and Sato (1965) and Hansel (1968) were seldom employed by other investigators.

In the present investigation mutation frequencies were estimated as the number of mutations per 100 M_1 plants, number of mutations per 100 M_1 spikes and number of mutants per 100 M_2 plants. The frequencies estimated as number of mutations per 100 M_1 plants gave higher values than the other two estimates at each of the doses employed in every mutagen. This evidently was an over estimation of the mutation event in consideration of the differentiated nature of the embryo. Mutation frequency on M_1 spike basis was estimated by scoring mutations on the apical and the primary axillary ears. The estimation of mutant frequencies on M_2 plant basis was made by scoring all M_2 plants in the progeny of these two ears. The frequencies estimated on M_1 spike basis and M_2 plant basis showed similar dose frequency relationships in almost every mutagen employed as would be clear from Figure 7. Thus, mutation frequency estimated as number of mutations per 100 M_1 spikes was found to be as efficient as the number of mutants per 100 M_2 plants

when the study was confined to the preformed ears.

For theoretical studies concerning mutation induction, it is very important to have a reliable system for comparison of mutation rates. Mutations for chlorophyll defects have been most widely employed for assessing the effectiveness of mutagenic treatments in higher plants (Nilan et al., 1963; Gaul, 1964a). In stressing their indispensability, Gaul (1964a) states that (i) they are the most frequent types of factor mutations, (ii) they can be readily recognised and classified, (iii) they can be studied in a small space under semi-controlled green-house conditions, and (iv) they provide rapid information as only seedlings need be grown. Walles(1963) reports that most of these mutants can synthesise some chlorophyll, but the pigment is destroyed to various degrees by photo-oxidation. Because of the defective chloroplast morphogenesis, Walles (1968) proposes that they should be called "chloroplast mutants" instead of chlorophyll mutants. However, the term chlorophyll mutants is retained herein to be in conformity with the terminology in general use in literature on mutation research.

The chlorophyll mutation frequencies estimated in the present investigation reached a maximum at very high doses with a decrease at the highest dose in each mutagen, thereby indicating an elimination of mutations at the highest doses. This decrease was conspicuous with mutagens such as gamma rays, fast neutrons, EMS and NMU which induced mutations with

high frequencies (Figure 7). Similar observations have been made by Matsuo et al. (1958), Maeima and Kawai (1959), Osone (1960), Yamaguchi (1964) and Mish et al. (1970) in rice. The dose-frequency relationship in the present study remained the same, irrespective of the method of estimation of frequencies, thereby indicating that the mutation frequencies decreased at the highest dose even when estimated on M_2 plant basis.

Among the relations employed, the highest frequencies of mutations were induced by gamma rays (Table XVIII). X-rays and fast neutrons induced mutations with almost similar but comparatively low frequencies. Fast neutrons, however, induced such frequencies of mutations at doses much lower than those of sparsely ionizing radiations. Matsuo and Onozawa (1961), Kawai (1968a), Mikaelsen et al. (1968), Mish et al. (1970) and Swaminathan et al. (1970) reported that neutrons induced a higher frequency of chlorophyll mutations at low doses.

Auerbach (1961) stated that alkylating mutagens were effective in all tested organisms. Ehrenberg (1960) and Nilan (1964) observed that the monofunctional alkylating agents were more efficient mutagens in barley, in that they produced a lower ratio of chromosomal aberrations to mutations than the bi-and polyfunctional agents. The five monofunctional alkylating agents employed in the present study showed differences in their mutagenic effects. EMS and NMU induced high frequencies of chlorophyll mutations in comparison to

the others. Similar findings were reported by Swaminathan (1966a), Siddiq (1967) and Ismail (1969) in rice. EMS was the most potent chemical mutagen employed in the present study and it induced a high frequency of mutations accompanied by low degree of M_1 damage. The highest frequency was 32.5 mutations per 100 M_1 spikes induced at the dose level of 288 mM. Konzak et al. (1965a) and Gaul et al. (1966) reported that EMS was the best among the chemical mutagens. However, the present results indicated that at equimolar concentrations MMS was more effective than EMS with respect to induced M_1 effects and M_2 mutations. For instance, MMS at 29.5 mM had reduced the seedling survival and height at 30 days to 70 and 80 per cent respectively, whereas, EMS at 38 mM did not induce any lethality or injury (Tables III & IV). Similarly MMS at 29.5 mM had induced 8.6 mutations as against 6.7 mutations per 100 M_1 spikes induced by EMS at 38 mM (Table VII). The high degree of effectiveness of MMS over EMS was reported by Heslot et al. (1959), Ehrenberg et al. (1961) and Froese-Gertzen et al. (1964).

In the present experiments, NMU induced high frequencies of mutations and ranked only next to EMS. The maximum frequencies of chlorophyll mutations per 100 M_1 spikes induced by EMS and NMU were 32.5 and 29.5 respectively. But at doses inducing high mutation frequencies, NMU induced very high lethality and injury thus revealing its high toxicity on the biological system. Though in respect of maximum frequency of mutations induced EMS was better than NMU, at equimolar

concentrations NMU was more effective than EMS. For instance, NMU at 7.76 mM induced 29.5 mutations as against 5.0 mutations per 100 M_1 spikes induced by EMS at 19 mM. The high mutagenicity of NMU was also reported in rice (Siddiq, 1967; Siddiq and Swaminathan, 1968a; Singh, 1970), wheat (Prasad, 1968), Arabidopsis (Gichner et al., 1969) and peas (Sharma and Rapoport, 1966). Among the different nitrosamides tested in barley by Ehrenberg and Gichner (1967), NMU was found to be the best, giving mutation rates nearly as high as those obtained with highly efficient alkane sulphonic esters.

The mutagenicity of MNNG was found to be very low in the present study. The highest frequency induced was 5.1 mutations per 100 M_1 spikes at 6.8 mM. Similar findings were reported by Ehrenberg and Gichner (1967) in barley and by Prasad (1968) in wheat. On the other hand, MNNG was reported to be an effective mutagen in Arabidopsis (Muller and Gichner, 1964; Muller, 1965; Gichner, 1965; Gichner and Veleminsky, 1967). Swaminathan et al. (1968, 1970) reported that MNNG was highly effective in rice. Thus, with respect to its mutagenicity, the available reports are at variance. A differential action of NMU and MNNG was observed in the present study. NMU was highly mutagenic whereas MNNG was not so. Similar findings have been made in barley by Veleminsky et al. (1967) and Gichner and Veleminsky (1967). In Arabidopsis, however, both were mutagenic and this was explained by Veleminsky and Gichner (1968) to be due to the effect of

hydroxylases present in Arabidopsis seeds which hydroxylated MNNG to diazo alkanes or carbonium ions, which in turn were strongly mutagenic. Heslot (1970) states that the nitroso compounds are indirect alkylating agents which alkylate through diazo derivatives.

Very low mutagenic effects were observed for DES in the present investigation. A maximum frequency of 8.9 mutations per 100 M_1 spikes was induced at 57.0 mM. Rao and Ayengar (1964) in rice and Heiner et al. (1960) in barley had reported that DES was very effective. A low rate of penetration might be responsible for the low mutagenicity of the fast reacting DES, in the present study as only seeds with hull were used. Konzak et al. (1965b) have stated that in barley seeds, the hull has been shown to reduce the effectiveness of treatments with DES.

The highest frequencies of chlorophyll mutations obtained in the present investigation with radiations, EMS and NMU compare favourably with the maximum frequencies reported in rice by earlier workers as compiled by Siddiq (1967). However, a comparison of these with the maximum frequencies for barley compiled by Nilan (1964) and Konzak et al. (1965a) gives an altogether different picture. Radiations are equally effective in rice as well as barley, whereas chemical mutagens induce much lower frequencies of mutations in rice than in barley.

Viable mutation frequencies were found to increase with increasing doses of mutagens reaching a maximum at or near the highest doses employed. In terms of maximum frequencies of mutations induced the mutagens could be arranged in decreasing order of potency as gamma rays, EMS, NMU and fast neutrons (Table XVIII) but at doses inducing similar biological effects viz., LD_{20} for injury they were in the order gamma rays, EMS, fast neutrons and NMU (Table XIX). Thus, irrespective of the criteria for efficiency gamma rays and EMS appeared to be efficient mutagens in inducing viable mutations. However, per unit dose, fast neutrons were most effective than gamma rays and at equimolar concentrations NMU was more effective than EMS. Siddiq (1967) reported that at comparable doses of EMS and gamma rays the frequencies of viable mutations were more or less the same in rice.

The comparison of physical and chemical mutagens is often made on the basis of M_2 mutation frequencies, since it is desired to realise the maximum frequency of mutations. Ehrenberg (1960) stated that the highest mutation frequency could be adopted as the best measure of mutagen efficiency. In the present investigation, a comparison of the maximum frequencies of mutations revealed EMS to be the best mutagen with respect to induced chlorophyll mutation frequencies, whereas, with respect to viable and total mutations, gamma rays were the best as could be seen from Table XVIII. But when compared at LD_{20} for injury, EMS was the best with

respect to chlorophyll and total mutations and gamma rays were the best with respect to viable mutations (Table XIX). These results indicate that while EMS induces more of chlorophyll mutations in rice, gamma rays induce more of viable mutations. Swaminathan (1966a) and Siddiq and Swaminathan (1968a) have reported that in rice gamma rays are as effective as EMS. Swaminathan et al. (1970) concludes that chemicals have no particular advantage over ionising radiations with reference to mutation frequency.

A detailed treatment of the concepts of mutagenic effectiveness and efficiency was presented by Konzak et al. (1965a). They proposed the terms "effectiveness" as a measure of gene mutations in relation to dose and "efficiency" as an estimate of the mutation rate in relation to other biological effects induced, such as lethality, injury or sterility. To obtain high efficiency, the mutagenic effect must greatly surpass other effects in the cell such as, chromosomal aberrations and toxic effects which generally lead to damage.

In the present study, radiations were found to be more effective than chemical mutagens and among radiations, fast neutrons were the most effective with respect to the induction of both chlorophyll and viable mutations. Among chemical mutagens MMU was the most effective. The high effectiveness of fast neutrons was reported by Siddiq (1967), Siddiq and Swaminathan (1968a), Swaminathan (1969b), and

Swaminathan et al. (1970) in rice and by Swaminathan (1965) and Shkvarnikov et al. (1966) in bread wheat. Siddiq (1967) reported that the most effective chemical mutagen in rice was NMU. In the present study, effectiveness was found to decrease with increasing doses of all mutagens. This inverse relationship was reported by several investigators in rice and could be explained as due to the failure of mutations to increase proportionately with increasing doses.

Mutagenic efficiency was higher for radiations when estimated on the basis of lethality and injury, whereas based on sterility chemical mutagens were highly efficient. Among radiations, fast neutrons were markedly efficient with respect to induction of both chlorophyll and viable mutations. The increased efficiency of neutrons was reported by Bhatia and Swaminathan (1963), Gopal-Ayengar and Swaminathan (1964) and Rana and Swaminathan (1967) in bread wheat and by D'Amato et al. (1962) and Scarascia-Mugnozza et al. (1963) in durum wheat. Among the chemical mutagens studied herein, EMS was the most efficient based on lethality and injury, but on the basis of sterility NMU was more efficient than EMS. The high mutagenic efficiency of EMS was repeatedly confirmed by several investigators in rice, barley, maize, Arabidopsis and in other plants. The superiority of EMS was probably because of its low phytotoxic effects on survival and inhibitory effects on seedling height. With radiations and chemicals except EMS, mutagenic efficiency decreased with increasing

doses, whereas with EMS, efficiency either remained the same or increased with doses. Siddiq (1967) reported that efficiency was higher at the middle doses with EMS whereas, with other mutagens efficiency was high at the lower doses. According to Konzak et al. (1965a), the greater efficiency of low doses of mutagens appeared to relate to the fact that injury, lethality and sterility increased with increase in mutagen concentrations at faster rates than mutations.

The usefulness of any mutagen depends on its mutagenic effectiveness as well as efficiency. The most effective mutagen need not be the most efficient one (Konzak et al., 1965a). In the present study, fast neutrons were found to be the most effective as well as efficient among the radiations. Among chemical mutagens NMU was the most effective but EMS was the most efficient. NMU can be a more efficient mutagen provided its toxicity is reduced by appropriate methods.

A high correlation between the frequencies of chlorophyll and viable mutations was obtained in treatment with fast neutrons, EMS and NMU in the present investigation (Figure 42). Matsuo and Onozawa (1961), and Sato (1966) in rice, Nilan (1961), Nagaraja Rao and Natarajan (1965), Ramanna and Natarajan (1965) and Doll and Sandfaer (1969) in barley and Chopra and Swaminathan (1966) in durum wheat also made similar conclusions. On the other hand, higher frequencies of viable mutations than those of chlorophyll mutations were induced by gamma rays in the present study. Gaul (1964a, b) and

Swaminathan (1965) stated that the proportion of progressive to chlorophyll mutations can be altered by changing the mutagen. A frequent occurrence of chlorophyll and viable mutations in the same panicle primordium was observed in treatments with EMS at higher doses, but not in treatments with gamma rays, fast neutrons and NMU in the present investigation (Table XV). Similar observations were made by Matsuo and Onozawa (1961) in rice with radiations and diepoxy butane and by Bremer--Reinders (1964, 1965) in Phalaris with X-rays and EMS. On the other hand, Siddiq (1967) did not find any such relationship and concluded that the manifestation of chlorophyll and viable mutations was independent of each other.

The mean segregation ratios of chlorophyll mutations increased with an increase in dose following treatment with radiations and chemical mutagens (Table X). The dependence of M_2 segregation ratio on dose was reported by Bekendam (1961), Yamaguchi (1962a), Kawai and Sato (1966) and Siddiq (1968) in rice, by Gaul (1961a, 1964a), Sarvella et al. (1962) and Aastveit (1968) in barley and by D'Amato (1965) and D'Amato et al. (1962) in durum wheat. The increase in the segregation ratio of mutants with increase in the dose of mutagens could be due to an increase in the size of the mutated sector resulting from lethality of some of the initial cells of the primordium as explained by Sarvella et al. (1962) and Aastveit (1968). The segregation ratio approached 25 per cent in treatments with high doses of gamma rays and X-rays which indicated that the mutated

sector occupied the entire ear. The generative tissue of the ear in this case might have developed from a single initial cell.

The mean segregation ratios however, were higher after treatment with radiations than with chemical mutagens. Similar observations were made by Kawai and Sato (1965) in rice, and by D'Amato (1965) and Scarascia-Mugnozza (1967) in durum wheat. The higher segregation ratio in treatment with radiations than with chemical mutagens indicated severe elimination of initial cells in the spike primordia following irradiation. Scarascia-Mugnozza et al. (1963) and Swaminathan et al. (1968), also experienced the same feature in the material they studied. However, high segregation ratio with MNNG as reported by Swaminathan (1969a) and a higher ratio for NMU than EMS as stated by Swaminathan et al. (1968) and Sevin et al. (1968) were not realised in the present studies. With high segregation ratios a high proportion of mutants will be observed, facilitating their selection in segregating populations. The increase in segregation ratio obtainable with a change of mutagen or with an increase in dose will no doubt be of immense value in mutation breeding. From the present studies, it is evident that in securing such high segregation ratios in rice, the radiations at higher doses appear to be more advantageous than the chemical mutagens.

Two mutations induced simultaneously in an ear primordium can be identified separately if they have different phenotypes. In the present investigation multiple chlorophyll

and viable mutations were more frequently induced by EMS at higher doses than by other mutagens. For instance, the frequency of ear-progenies with two mutations increased progressively with increasing doses of EMS and those yielding three types of mutations were regularly seen only at the high doses (Tables IX and XIII). Such an increase in the frequency of multiple mutations following treatment with chemical mutagens was previously reported by Siddiq (1967) in rice, and Savin et al. (1968) and Sharma (1970) in barley. Hansel (1967) also reported an increase in the frequency of multiple mutations in barley with increasing doses of EMS.

Kawai and Sato (1965) and Hansel (1967) report that multiple mutations arise as two mutations induced either in the same initial cell or in different initial cells of the same spike. In the former type (true multiple mutations) the M_2 progeny will contain three mutant types viz., the two types representing the individual effects of the two mutated genes and a third type representing the double recessive mutant genotype. When two mutations are induced in different initial cells, the double mutant will not appear in the M_2 generation. Four cases of true multiple mutations were observed following treatment with EMS and NMU, in the present study. In each of these cases, three types of mutants were isolated from the M_2 progeny. The ear-progenies being small in size, the frequencies of mutants did not give a dihybrid ratio in the M_2 generation. However, the segregation patterns in the M_3 progeny of normal M_2 plants confirmed that

two mutants were monogenic recessives representing the two mutated genes and the third mutant was a double recessive of the two.

Correlation of M_1 effects and M2 chlorophyll mutation frequencies

In the present investigation, M_1 chlorophyll chimeras were observed following treatment with NMU, EMS and fast neutrons, but not in treatments with gamma rays in spite of its high mutagenicity. Therefore, the frequency of chlorophyll chimeras did not provide an indication on the relative mutagenic effects of radiations and chemical mutagens. A comparison of EMS and NMU at the doses 384 mM and 2.91 mM respectively inducing similar frequencies of M_1 chimeras had indicated that the chlorophyll mutation frequency with EMS was more than double that of NMU. Thus, even for a comparison of the mutagenic effects of chemical mutagens the frequencies of M_1 chlorophyll chimeras did not provide a reliable criterion. The incidence of chlorotic streaks might be associated with the reaction mechanism of individual chemical mutagens. In mutagens such as NMU and EMS, which induce them readily, their frequencies may be useful in studying dose effect relationships. Wettstein (1961), D'Amato et al.(1962) and Naterajan and Shivasankar (1965) did not find a positive correlation between the frequencies of M_1 chlorophyll chimeras and M_2 mutations. On the other hand, Heslot (1961) and Savin et al. (1968) in barley, MacKey (1965) in wheat and Klixt et al. (1960, 1963) in peas reported high correlations.

Goud (1963), Varughese (1966), Varughese and Swaminathan (1968) and Prabhakara Rao and Washington (1970) reported that the chlorophyll chimeras did not breed true. The sectors on leaves were reported to originate through somatic mutations as periclinal chimeras, probably in the outer L_I layer and since the germinal tissue develops from the inner L_{II} layer, the mutations might not pass on to the progeny (D'Amato et al., 1962). In the present investigation, the chimeric tillers were found to yield mutations in their progeny more frequently than the non-chimeric tillers (Table XXI). It was also observed that chlorophyll deficient sectors on leaves and panicles were of independent origin and that the striped panicles yield mutants more frequently than normal panicles produced by chimeric tillers. Thus, it is evident that sectors on the panicles and spikelets give a more sure indication of mutations in the progeny than the presence of sectors on the leaves. It may therefore, be possible to realise a high frequency of mutations in the M_2 generation by selecting M_1 panicles with chlorophyll deficient sectors.

It is important to know from a practical breeding point of view, the interrelation of fertility in the M_1 generation and mutation frequency in the M_2 generation. In the present investigation when M_1 ears were selected at random, mutation frequency was found to increase with decreasing seed fertility following treatment with gamma rays as well as EMS, but at the lowest fertility level the frequencies decreased (Table XXIV). Thus, the frequency of mutations was low at the

highest and lowest fertility levels. There was no apparent difference between gamma irradiation and EMS treatment in this respect as may be seen from Figure 43-A. The decrease in the lowest fertility class might be due to elimination of mutants and that at the highest class might be due to either a low degree of induction or diplontic selection. Similar results were reported by Eskendam (1961) in rice following X-irradiation. D'Amato (1962), D'Amato et al. (1962) and Scarascia-Mugnozza et al. (1963) observed in durum wheat significant negative correlation after treatment with ionising radiations, while in chemical mutagen treatments, mutations accumulated towards higher fertility classes. Significant negative correlation between seed fertility and M_2 mutation rates was reported by Ramanna and Natarajan (1965) and Nagaraja Rao and Natarajan (1965) in barley, by Hildering and Van Der Veen (1966) and Van Der Veen and Hildering (1966) in tomato, by Wellensiek (1965) in peas and by Merker (1970) in Lathyrus.

On the other hand, Gaul (1961a, 1965, 1970), Gaul and Mittelstenscheid (1960) and Gaul et al. (1969) in barley, Rana and Swaminathan (1967) in bread wheat and Muller (1967) in Arabidopsis found that the frequency of M_2 chlorophyll mutations was independent of the degree of M_1 sterility. This was explained to be due to the independent induction of chromosomal aberrations and gene mutations in the M_1 plant. Gaul (1964a, 1965) however,

suggested that the independence of M_1 sterility and M_2 mutations would hold good only when the degree of tillering in the M_1 plant was limited and the preformed spikes alone were considered. An analysis of the association between sterility and mutation frequency in the preformed and postformed ears of the M_1 plant was not taken up in the present study.

Specificity of mutagens and mutation spectrum

Nilan et al. (1965) stated that the alteration of the spectrum of chlorophyll mutants provided evidence for mutagen specificity in higher plants. Nilan (1967) in reviewing the various reports of alteration in spectrum of mutations induced by different mutagens has concluded that a precise control over spectrum is yet to be achieved. As pointed out by Auerbach (1961) mutagen specificity from a breeding point of view is phenotypical. The vast majority of induced mutations are harmful and therefore, means for directing mutations into desirable channels would be of great importance for mutation breeding (Auerbach, 1967).

Favret (1960) and Ryan and Heslot (1963) clearly demonstrated the randomness in action of radiations as well as specificity of EMS to certain loci in barley. According to Auerbach (1961) and Smith (1961) the specificity of action of chemical mutagens was regional than genic. In general, after treatment with chemicals more breaks were found in the heterochromatin, near the centromere and at

constriction regions (Gaul, 1964a, 1970; Gustafsson, 1969). Swaminathan et al. (1962) suggested that the location of genes relating to chlorophyll development in the proximal segments and the high susceptibility of such regions to EMS action might be the factors involved in the induction of a large number of chlorophyll mutants with EMS. The localisation of chlorophyll development genes near the centromere observed in barley by Robertson (1964) and Nilan (1964) and in maize by Neuffer (1966) supported this suggestion. As stated earlier the frequency of chlorophyll mutations induced in treatments with EMS was considerably higher than in case of other mutagens in the present study. The specificity of EMS in this respect as indicated by earlier workers had become evident in rice also.

The results obtained in the present investigation reveal that the spectrum of induced mutants differ between radiations and chemical mutagens (Figure 8). Albina was the most frequent type following treatment with radiations. In chemical mutagen treatments there was a decrease in albina followed by an increase in the frequency of viridis. Such significant differences in the spectrum of mutants between radiations on the one hand and chemical mutagens on the other were reported by Bekendam (1961), Cheo and Chai (1961), Katsumura and Mabuchi (1964), Nawai (1966) and Basu and Basu (1969) in rice, by Ehrenberg et al. (1956), Nilan and Konzak (1961), Nilan et al. (1964), Swaminathan et al. (1962), Gustafsson (1963), Konzak et al.

(1965a) and Doll and Sandfaer (1969) in barley, by D'Amato (1962), D'Amato et al. (1962), Scarascia-Mugnozza (1967) and Scarascia-Mugnozza et al. (1963) in durum wheat and by Swaminathan et al. (1962) and Goud (1967a) in bread wheat. Differences in the spectrum of mutations were not significant between doses of either radiations or chemical mutagens in the present study. Gaul (1964a) and Kawai and Sato (1965) also reported similar results.

There are several reports of differences in the spectrum of viable morphological mutations induced by radiations and chemical mutagens. The strongest evidence comes from the erectoides mutants in barley. In the present experiments with rice the types of viable mutations induced with the different mutagens did not differ, but their relative frequencies were different (Table XII). Erectoides mutants were frequently induced with fast neutrons and NMU. Ehrenberg et al. (1961) and Nilan et al. (1965) reported a high rate of erectoides mutants induced by neutrons in barley. It was also evident from the analysis of the spectrum of mutations induced in the present study, that high frequencies of mutants with altered duration and grain type following treatment with gamma rays, mutants affecting culm length and mutants with panicle and spikelet abnormalities with EMS and fast neutron treatments respectively could be realised. These observations suggested that the mutation spectrum could be altered through the use of different mutagens.

Types of viable mutants

A large number of viable mutants were isolated in the present study. They appeared in low frequencies in segregating ear-progenies in the M_2 generation and bred true in the succeeding generations. Their first appearance in the M_2 generation and their pure breeding nature had clearly indicated that each of them represented changes in a single recessive gene from the parent variety. Gaul (1964a), Nilan (1964), Tuleen et al. (1966) and Moh (1968) stated that most of the mutations in diploid organisms were recessive. Several investigators had reported the monogenic recessive nature of induced mutations in rice (Hsieh, 1962; Butany and Battacharya, 1962; Yamaguchi 1964; Gill et al., 1969). Three double recessive mutants were isolated from M_2 lines segregating for multiple mutations. Their double recessive nature was confirmed when certain of the normal M_2 plants segregated in the M_3 generation in the dihybrid ratio. An instance of incomplete dominance was recorded when a fine grain mutant segregated in the M_3 generation in the 1:2:1 ratio. The segregation was tested and confirmed in the M_4 generation. The heterozygous genotype produced fine grains whereas, the homozygous mutant gene produced abnormal spikelets with elongated lemma (Figure 37-5). Mutants with incomplete dominance were reported by Tuleen et al. (1966) and Tsuchiya (1969) in barley and by D'Amato (1962) and D'Amato et al. (1962, 1964) in durum wheat. Two viable striata mutants were found to be inherited maternally indicating that they were

governed by mutated plastids. Maternal inheritance of chlorophyll deficiency through plastids have been reported in rice by Morinaga (1932) and Ramiah and Ramanujam (1935). Gene induced plastid mutations were reported by Pal and Ramanujam (1941) in rice and by Stubbe (1967) in barley.

Macro-mutants: Macro-mutants including several erectoides types formed the predominant group of viable mutants isolated in the present study. The erectoides mutants were characterised by thick culm, short broad leaves which were stiff and coarse and dark green in colour, and by the exerted compact stiff panicles and small or medium sized bold grains (Figures 12 to 14). Most of them were either dwarf or semi-dwarf in stature. The different types of erectoides mutants were found to differ with respect to characters such as height, stiffness of culm and leaves, number of tillers, compactness of panicles and size of grains. They exhibited more variability in panicle and grain characters than in culm and leaf characters. Erectoides mutants were frequently reported in experiments on induced mutagenesis in cereals such as rice (Nesiam and Kawai, 1958; Hu et al., 1960; Matsuo and Onozawa, 1961; Li et al., 1961, 1962, 1966a; Kawai, 1968b), barley, wheat and oats. Persson and Hagberg (1969) stated that in barley the erectoides mutants were more commonly induced than any other type of viable morphological mutants. The constellation of characters distinguishing each erectoides mutant was found in the present investigation to be governed by a single

recessive gene. There was no evidence of recombination of the characters belonging to an *erectoides* complex in the M_2 progeny which segregated for such mutants. Based on phenotypic differences, 20 *erectoides* mutants were recognised. They might be governed by different gene loci as well as by multiple alleles at the same locus. Li *et al.* (1965, 1966a, b, 1968) and Hu *et al.* (1970) had reported that the induced *erectoides* mutants in rice were controlled by the same locus with a multiple allelic series. Gustafsson *et al.* (1969) stated that the 182 localised *erectoides* mutants out of 685 reported in barley could be assigned to 26 gene loci.

Several macro-mutants other than *erectoides* were isolated in the present investigation. These mutants also showed alteration in a number of characters simultaneously (Figures 15 to 19). Most of them were either dwarf or semi-dwarf in stature. Alterations in plant height and grain type were more frequent (Figure 24). The complex of characters expressed by these macro-mutants were inherited as a single genetic unit indicating that they were governed by simple recessive genes. The macro-mutants exhibited differences between themselves with respect to almost every character thereby indicating that they were governed by a number of gene loci.

The simultaneous effects of macro-mutations on almost every character of the plant has been explained by many investigators to be due to pleiotropic action of a single

mutated gene. Gottschalk (1968, 1970) suggested three possible interpretations for the multiple effects in mutated organisms, viz., (1) single mutated gene being responsible for the whole complex of deviating characters, (2) a tiny portion of a chromosome containing several genes being lost, or (3) several closely linked genes having mutated. The first case is genuine pleiotropism, whereas the other two events simulate the pleiotropic effect of one gene although several genes are lost or altered. Stubbe (1959) in snapdragon, Williams (1960) in tomato, Gottschalk (1965, 1968) in peas and Hesemann and Gaul (1967) and Gaul et al. (1968) in barley have demonstrated the possibility of dissolving the pleiotropic spectrum of a mutation into the component effects, thereby indicating that the macro-mutants represented mutations in tightly linked genes.

Visible mutants: Mutations affecting various individual characters, such as height of plants, duration, type of leaf, ear and grain were isolated in the present investigation. Mutants with variability in plant height were more frequent than those exhibiting diversity in other characters. Most of the changes were towards shorter culm length. The dwarf and semi-dwarf mutants without pleiotropic effect on the panicle and grain were found to represent different grades of height reduction from the parent variety. The tall mutants represented alteration of plant height in the positive direction (Figures 27 & 28). It was evident that when recessive alleles at certain loci controlled short stature, recessives at certain other loci governed tall stature.

Kawai (1962) and Tanaka (1960) reported that in rice, mutants with reduced height were of more common occurrence than those with increased height. Konzak (1966) also reported that short culm mutations were readily induced in common wheat, durum wheat and barley.

Mutants with altered maturity period were more frequently of late types than early ones. The frequent changes tending towards lateness had indicated that recessive mutations in an early variety had altered the maturity period towards lateness. The several mutants affecting duration obtained in the present study consisted of a complete array of types ranging from very early (85 days) to very late ones (180 days) against the maturity period of 120 days for the parent variety (Figures 29 to 31). Kawai (1962, 1963a) and Sato (1966) reported that lateness was more frequently induced than earliness, and Yamagata (1964) and Matsuo and Yamaguchi (1967) postulated that a high frequency of late mutants would be induced in an early variety.

Several mutants with alterations in grain size were recorded in the present investigation. There were types with small, medium and large grains and others with bold or fine grains. Mutants with narrow leaves and long ears were also found to arise occasionally (Figures 32 to 34).

Mutants with defective panicle and spikelet development were also observed. Induced panicle abnormalities included

types with accessory parts such as neck leaf, with incomplete development of the rachis or the primary branches and extreme clustering of spikelets (Figure 35). The abnormal spikelet types were represented by those with extreme reduction of fertile glumes, with glumes modified into awn and with differential elongation of lemma and palea. In certain types the sterile glumes were highly elongated (Figures 36 to 39). In several cases, the spikelet abnormalities were associated with abnormal floret development leading to total sterility. Even in mutants with normal florets, the grains did not develop properly due to defective fertile glumes. Mutants with spikelet abnormality of a low intensity were found to produce fertile grains of different sizes. Genetically controlled errors in the development and organisation of floral structures were reported in rice by several workers (Ramiah and Parthasarathy, 1938; Kadam et al., 1941; Nayaheri and Bora, 1963; Oka, 1963; Siddiq, 1967; Gill et al., 1969; Ganeshan, 1970; Swaminathan et al., 1970). Kamra (1966) reported several mutants with transformed floral organs in barley induced with radiations and chemical mutagens. Thus, the present study on viable mutations indicate that most of the morphological features can be altered by induced mutagenesis.

Systematic mutants: The systematic mutants belong to the category of macro-mutants in that they represent changes in a constellation of characters from the parent variety. The M_2 progenies which yielded these mutants in the present study did not segregate for other types and the entire phenotype was

inherited as a single genetic unit. Thus, they represented single gene differences from the parent variety, thereby indicating that the key characters controlling species and subspecies differences could be decided by individual genes. These mutants were found to deviate drastically from the parent variety with respect to characters such as plant height, thickness of culm, number of tillers, length and colour of leaves, type of ear and grain, heariness of grains and fertility. Such drastic changes in respect of the important plant characters were not seen among the varieties of Oryza sativa and therefore, these mutants might be considered to have crossed the limits of variability generally recognised for varieties within the species. Two of them were found to be similar to O.perennis subsp. barthii by virtue of their tall habit, thick culm, dark and coarse leaves and awned hairy grains (Figure 40—2&3). The third mutant was dwarf in stature with high tillering habit, thin culm and short open panicles with small, awned grains (Figure 41—1). The characteristics of the ear and grain in this mutant were similar to O.sativa v.fatua. Systematic mutants have been reported by Shastry (1965) and Swaminathan (1966b) in rice, by MacKey (1954), Swaminathan (1963, 1966c), Swaminathan et al. (1963) and Bozzini (1964) in hexaploid wheat, by Scarascia-Mugnozza et al. (1963), D'Amato et al. (1964), Kuckuck and Peters (1964), Budashkina and Stchapova (1966) and Upadhya and Swaminathan (1969) in tetraploid wheat, by Schmalz (1962) in barley and by Gottschalk (1969) in

legumes. Swaminathan (1965) succeeded in synthesising all the known subspecies of Triticum aestivum from one variety of subspecies vulgare. The tool of induced mutations had been very elegantly used to understand the process of evolution and phylogeny by Stubbe (1959, 1967) in barley, tomato and snapdragon, by Swaminathan (1966c) in hexaploid wheat and by Siddiq and Swaminathan (1968b) in rice.

Germplasm of mutants

The 421 viable mutants isolated in the present study represented variability for almost every character of the rice plant. The variability met with in these mutants was quite impressive with respect to all morphological characters in general and for height of plants, duration and size of grains in particular. This had indicated the scope for altering any character by adopting the technique of induced mutations. Many of these types were macro-mutants in which a desirable change in one character was associated with an undesirable change in another, thereby rendering the mutant unsuitable for direct use as an improved variety. However, they could be of immense value in breeding programmes where the association between characters could be dissolved by appropriate techniques. It would be possible to either separate the pleiotropic effects by inducing recombination or alter the expression of macro--mutant traits by changing the genetic background in which the genes were located. The visible mutants representing changes in individual characters also revealed enormous variability that could be utilised for improvement of rice.

Several investigators had reported that the variation found in a collection of induced mutants corresponded to the diversity of characters present in a world collection of varieties (Nilan, 1964; Stubbe, 1967; Hagberg and Persson, 1968). Swaminathan (1965) stated that it would be possible to artificially generate a large variability in a single variety of wheat or barley and Scholz (1967) succeeded in demonstrating this possibility, a reality in barley. Collection of mutants in barley maintained in Sweden (Gustafsson et al., 1969) in Germany and Belgium (Nilan, 1964; Gaul, 1964a), in Hungary, Rumania and Canada (Nilan, 1964) and Japan (Tsuchiya, 1969), in durum wheat (D'Amato, 1962), in peas (Gottschalk, 1969), in snapdragon, soybeans and tomato (Stubbe, 1967) is well known. Large collection of mutants in rice was reported by Ekendam (1961), Kawai (1968b) and Tanaka (1969) in the subspecies japonica. The existence of such a collection had not been made known in subsp. indica. A modest collection of mutants, had however, been made possible by the present study.

III. Attempts for enhancing mutagenic efficiency

Combination treatments

One of the methods of increasing mutation frequency is by combining treatments with two or more mutagens. This approach makes use of the fact that various physical and chemical mutagens induce different spectra of mutations. Thus, combination of two mutagens will increase the mutation frequency and enlarge the mutation spectrum. In the two combination treatments of gamma rays with NMU and fast neutrons

with NMU employed in the present study, the effects of the mutagens on survival and seedling height in the M_1 generation were additive at all dose combinations (Table XXIX—i&ii). Soriano (1968) found that EMS when applied after irradiation of rice seeds with fast neutrons did not affect seedling height and fertility. Prasad (1968) observed that NMU in combination with gamma rays acted effectively in wheat, while such synergism was not observed in the case of gamma rays with EMS. Mutation frequencies in the M_2 generation induced by the combination treatments employed in the present investigation were not higher than those expected from simple additive effects of the mutagens when estimated as the number of mutations per 100 M_1 spikes. But the frequencies realised as number of mutants per 100 M_2 plants were higher after combination treatments than the sum total of frequencies, after single treatments. The synergistic effect was most pronounced at the combinations of high doses of fast neutrons and NMU and this was substantiated by the high segregation ratio of mutants (Table XXIX—iii to v). Thus, the higher dose combinations of fast neutrons and NMU were very efficient in terms of mutant frequency. Aastveit (1968) and Sharma and Swaminathan (1970) in combination treatments of gamma rays and EMS in barley observed similar synergistic effects on mutant frequencies.

The synergistic effects in combination treatments of mutagens are explained in sevel ways. Scarascia-Mugnozza (1969) states that if one mutagen sensitises previously

protected sites to the second mutagen, more than additive effects can be expected from sequential application of mutagens. Inactivation of repair enzymes by the second mutagen will increase the chances of potential mutational lesions induced by the first mutagen to get fixed (Sharma and Swaminathan, 1970). Bhatia (1970) states that radiations induce changes in the properties of biological membranes and this will enhance the penetration of the chemical mutagen when it is given following irradiation, leading to synergistic effects.

Recurrent and alternate treatments

Repeated treatments of seeds with mutagens in successive generations is a method of accumulating mutations. In the present study, the sensitivity of seeds collected from the M_1 population obtained from seeds irradiated with gamma rays, when subjected to gamma rays as well as NMU, as a second cycle of treatment, was not affected (Table XXXII-iii). Yamaguchi (1962b) obtained similar results in barley and rice after recurrent X-irradiation. Frydenberg and Sandfaer (1965) also observed that recurrent irradiation of barley with gamma rays revealed no differences in seed germinability and plant height. It was also observed in the present investigation that the mutation frequencies in the recurrent as well as alternate treatments were less than the sum total of frequencies of the individual treatments (Table XXXII—iii). If mutagens had exhibited specificity of action, the alternate treatment with NMU would have induced more mutations than

a recurrent dose of gamma rays, as explained in oats by Joshi and Frey (1967). Siddiq and Swaminathan (1968d) also reported a reduction in mutation frequency in the recurrently irradiated population of rice.

Polyploids, in general, were reported to respond favourably to recurrent irradiation through an increase in the frequency of mutations (Swaminathan, 1961, 1965; Gaul, 1964a). The absence of such an increase in the present study following recurrent and alternate treatments of mutagen, therefore, indicate that rice behaves like a diploid. Similar conclusions have been drawn by Siddiq and Swaminathan (1968d).

Presoaking of seeds before mutagenic treatments

Increase in sensitivity of seeds to radiations following presoaking has been demonstrated in different plants, since the early report of Stadler (1928a) that soaked barley seeds were more radiosensitive than dry seeds. Of late, due attention is being given to the problem of increased sensitivity of seeds to chemical mutagens following presoaking. In the present study, sensitivity of rice seeds to NMU increased progressively with increasing periods of presoaking and reached a maximum at 32 hour duration. There was a decrease in sensitivity when presoaking was extended beyond 32 hours as would be evident from Figure 44(i). The time specificity of the sensitivity peak was the same irrespective of the dose of the mutagen and the criteria adopted to estimate sensitivity. However, the intensity of damage was more at the higher dose and greater in terms of lethality than injury. Sensitivity was reported

to be greatly enhanced by presoaking seeds in water before treatment with chemical mutagens by Ando (1968), Ismail (1969), Gopal-Ayengar et al. (1969), Swaminathan et al. (1970), Siddiq et al. (1970) and Sharma Rao (1970) in rice, by Natarajan and Shivasankar (1965), Savin et al. (1968), Swaminathan et al. (1968) and Brunner et al. (1968) in barley, by Sree Ramulu (1970) in sorghum, by Blixt (1967) and Blixt and Gelin (1965) in peas, by Nerker (1970) in Lathyrus and by Robbelen (1965a and b) in Arabidopsis. In dehulled seeds of rice, Swaminathan et al. (1970) found a drastic increase in sensitivity to treatment with EMS after presoaking for 18 to 22 hours. Similarly, peak sensitivity to mutagen treatment was obtained in barley seeds presoaked for 16 hours (Natarajan and Shivasankar, 1965; Savin et al., 1968; Swaminathan et al., 1968), in maize seeds presoaked for 26 hours (Latteral, 1961) and in Arabidopsis seeds presoaked for 12 to 15 hours (Robbelen, 1965b). The specificity of presoaking period as related to mutagen sensitivity peaks irrespective of the criteria of damage, mutagen and its doses indicated the operation of an underlying common cause (Swaminathan et al, 1968).

The increase in sensitivity caused by presoaking was attributed to the leaching of endogenous protective substances (Kamra et al., 1960), Oxygen enrichment (Latteral, 1961), changes in general metabolic condition of the cells (Sharma, 1966) and progress in DNA synthesis (Natarajan and Shivasankar, 1965). Natarajan and Shivasankar (1965) postulated that the

first DNA synthesis taking place in the cell initials was the most important factor responsible for the increased sensitivity during the 16 to 18 hours presoaking period in barley seeds. Autoradiographic studies by Savin et al. (1968) have confirmed this hypothesis. In an indica variety of rice, autoradiographic studies by Gopal-Ayengar et al. (1969) have revealed that DNA synthesis is initiated between 24 and 32 hours in seeds with hull and between 12 and 16 hours in seeds without hull. Thus, the peak period of sensitivity to NMU observed in the present study corresponds to the period of DNA synthesis in the initial cells. In fact the increase in sensitivity is very sharp during the period from 20 to 32 hours (Figure 44—i). It is therefore, evident that the first DNA synthesis taking place in the cell initials is the most important factor responsible for the peak sensitivity at the 32 hours presoaking period. In dehulled rice seeds also the peak period of sensitivity reported by Swaminathan et al. (1970) corresponds to the period of DNA synthesis determined for that category of seeds.

The frequency of chlorophyll mutations increased with an increase in presoaking time reaching a maximum at 40 hours duration in the present investigation. The most conspicuous increases, however, were during the periods of 16 to 20 hours and 24 to 28 hours (Figure 44-ii). This enhanced efficiency during the 16 to 28 hour period might be attributed to the synchronisation of treatment time with the S phase of DNA synthesis. Gopal-Ayengar et al. (1969) also observed a

sharp rise in mutation frequencies during the 24 to 32 hour period and a further increase beyond 32 hours reaching a maximum at 48 hour period. The sudden increase in mutation frequency in seeds without hull observed by Ismail (1969) and Siddiq et al. (1970) also corresponded to the period of DNA synthesis for dehulled seeds. Natarajan and Shivasankar (1965), Savin et al. (1968), Swaminathan (1969a), Swaminathan et al. (1968) and Nilsolsen (1969) in barley and Robbelen (1965a and b) in Arabidopsis have also observed that the mutation frequency was the highest when the treatment was administered at the time of DNA synthesis.

The relative percentages of different types of chlorophyll mutants induced by NMU in the present study were altered by presoaking of seeds for various periods prior to treatment. Albinas were frequent at long periods of presoaking, while viridis mutants were numerically high at short duration of presoaking. Xanthas and tigrinas appeared in low frequencies only in seed lots presoaked for 20 hours and beyond, and the development of xanthas and tigrinas from such treated seed lots alone was the most characteristic feature of the chlorophyll mutant spectrum. An increase in the percentage of albins following treatment of seeds with EMS after presoaking was reported by Natarajan and Shivasankar (1965) in barley. The frequency of viridis mutants was high following treatment with NMU after short periods of presoaking in rice (Siddiq et al., 1970). In S phase fractionation experiments in barley, Swaminathan (1969a and b) and Swaminathan and

Sharma (1968) have reported a delayed appearance of xantha and tigrina mutants. However, the large number of loci governing chlorophyll mutants in barley was reported to offer a major difficulty in explaining these results. Gustafsson (1956) indicated that in barley nearly 250 to 300 loci were involved in chlorophyll synthesis of which 125 to 150 might be concerned with albina, 125 with viridis, 10 to 15 with xantha and 15 to 20 with rare types. The genetics of xantha determination was thus, relatively simple. In the present investigation employing different radiations and chemical mutagens, xantha and tigrina mutants were induced in low frequencies and this might indicate that these mutants were governed by low number of loci in the genotype of the rice plant. This relatively lesser number of loci concerned in the determination of xantha and tigrina mutants might account for the specificity observed with regard to the time dependence of their appearance.

Changing the spectrum of mutations in a predictable manner and thereby achieving directed mutagenesis is an important goal of current mutation research. Grant and Healot (1966) found that different chromosome regions exhibited cyclic changes in duplication and in sensitivity to mutagens and suggested that if cells were subjected to pulse treatment with a mutagen specifically affecting the chromosome under duplication, it could be expected that particular sites of the chromosomes would be affected and specific mutations would be induced. Cerda-Olmedo and Hanawalt (1968) found in Escherichia coli, treated with

MNNG that the maximum frequency of a given type of mutation occurred when the treatment was given at the time when the gene was in the process of replication. Swaminathan (1969a) concluded that since the DNA replication along a chromosome is asynchronous in time sequence, it would be possible to affect groups of loci preferentially by administering the treatment for short periods at different stages of the S phase. Thus, the relative frequency of different classes of mutants could be manipulated by synchronising the treatment with the time of replication of a specific locus. The alteration of mutation spectrum observed in the present investigation in treatment with NMU following presoaking for varying periods had indicated the scope for further investigation in this line, towards attaining specificity of mutations in rice.

IV. Problems related to the recovery of induced mutations

Success in mutation breeding will largely depend upon the adoption of suitable procedures for handling of mutagen treated material based on a knowledge of the factors influencing recovery of mutants. Induction of mutations at the level of the locus and their subsequent recovery are two independent processes. The extent to which a mutation induced by seed treatment may survive and give rise to a mutant is controlled by many factors, among which those relating to the biological characteristics of the seed such as the stage of differentiation of the embryo and the number of primordial cells involved in the origin of each panicle are very important. The embryo in rice is known to be differentiated into a number of primordia each

of which possessing the potential ability of giving rise to a panicle or a group of panicles. Each primordium in turn consists of a number of initial cells, the division products of which constitute the generative tissue of the panicle (Bekendam, 1961; Osone, 1963; Swaminathan 1966a). A panicle developing from two or more initial cells, of which one has mutated, represents an undetected sectorial chimera. The part containing the mutation is referred to as the mutated sector. The study of mutated sectors is important to calculate the M_2 progeny size needed to obtain the maximum frequency of mutants. The size of the sector can be estimated from the segregation ratio of mutants in the progeny.

The M_2 segregation ratio of a mutated panicle (ear) depends on the size of the mutated sector, the mode of inheritance of the induced mutation and the deleterious changes that might accompany the mutation leading to a deficit of recessives. D'Amato (1962, 1965) stated that in the case of monogenic recessive mutations and under self-pollination, if deleterious changes accompanying the mutations are excluded, the M_2 segregation ratios will be proportional to the size of the mutated sector of the M_1 ear. In the present investigation, several M_1 ear-progenies segregated with significant deficit of recessive mutants in the M_2 generation. This deficit of recessives might be due to one or more of the reasons such as (1) mutated sector in the M_1 ear, (2) mutant phenotype being governed by more than one gene, (3) haplontic selection resulting in the elimination of gametes carrying the mutated

gene, and (4) Diplontic elimination of recessive homozygous mutants at the zygote or proembryo stage. The ear-progenies were grouped into different categories by a comparison of the percentages of mutants and heterozygotes in the M_2 generation and mutants in the M_3 generation. Progenies segregating in a 3:1 ratio giving 25 per cent of recessive mutants in the M_3 generation but with deficit of mutants and heterozygotes in the M_2 generation were selected for the estimation of sector size. Certain progenies with deficit of recessives in the M_3 generation were also selected for this purpose because they recorded a proportionate reduction of mutants and heterozygotes in the M_2 generation. However, only 50 per cent of the progenies studied could be advanced for estimation of sector size. Others were unsuitable for the purpose since the segregation ratios in the M_2 and M_3 generations indicated the operation of factors other than sector formation leading to the deficit of recessives.

Mutated sector size

In the present investigation the sector size of each M_1 ear was estimated by dividing the M_2 segregation ratio by the M_3 ratio of the respective progeny. Kawai and Sato (1965) in rice and Beard (1970) in flax have adopted a similar procedure. On the other hand, Nekrasam (1961) and Osone (1963) in rice, Gaul (1961a) in barley, and D'Amato et al. (1962) and Scarascia-Mugnozza et al. (1963) in durum wheat assumed the M_3 ratio as 20 per cent based on the report of Noh and Smith (1951) in wheat and barley in which the average

frequency of recessive chlorophyll mutants was estimated as 20 per cent. Siddiq (1968) assumed the M_3 ratio for rice as 25 per cent. The present results however, indicated that several progenies segregated in the M_3 generation with recessive deficits of different magnitudes, the M_3 ratios ranging from 3.8 to 28.2 per cent (Tables XXXIX to XLVI). Therefore, the segregation ratio in the M_3 generation of a progeny was taken as the basis for estimation of the sector size of the concerned M_1 ear. Mutated sector size was estimated by scoring of sectors of aborted pollen in maize (Anderson et al., 1949), by scoring of waxy mutants in barley (Eriksson, 1965, 1967), by the topographical method of locating mutants on ears in durum wheat (D'Amato, 1965) and by carrying out Chi square analysis for fitness of the expected M_2 segregation ratios in peas (Weiling and Gottschalk, 1961; Monti, 1966).

The size of the mutated sector estimated in the present investigation was found to differ considerably from ear to ear. The sectors induced by chemical mutagens were smaller in size than those induced by radiations (Tables XXXIX to XLII). Similar observations were made by Kawai and Sato (1965) and Siddiq (1968) in rice, by Gaul (1964a), Lindgren et al. (1970) and Eriksson and Lindgren (1970) in barley and by D'Amato (1962) and D'Amato et al. (1962) in durum wheat. A difference in the mutagenic action of radiations and EMS was reported to be the cause of the difference in sector size. The small size of the mutated sector following chemical mutagen treatments was explained by Ehrenberg (1965), D'Amato (1965), Kawai and Sato (1969)

and Lindgren et al. (1970) to be due to the delayed action of chemicals. Auerbach (1964) stated that chemical mutagens had a tendency to produce mutations and chromosomal breaks after a delay that might extend over many cell generations. Lindgren et al. (1970) concluded that because of the delayed action following EMS treatment only gamma ray-induced sectors were suitable for the estimation of sector size.

The mutated sectors in the present study were found to be comparatively smaller in the apical ear than in the primary axillary ear, thereby indicating that a larger number of meristematic initial cells were involved in the formation of the apical ears than in the primary axillary ones. Similar conclusions were drawn by D'Amato (1965).

Number of initial cells

The number of primordial cells involved in the origin will govern the size of the mutated sector of the ear. The number of effective initial cells contributing to the formation of the generative tissue of each M_1 ear was estimated in the present study as the reciprocal of the sector size. This estimation is based on the assumption that the cells carrying the mutations and the normal cells divide at the same rate. If the cells with mutation divide less frequently than the normal cells, the result will be an over-estimation of the number of initials. Frydenberg et al. (1964) in barley and Muller (1965) in Arabidopsis reported that the mutated sector had no developmental advantage or

disadvantage in comparison with the non-mutated sector. Lindgren et al. (1970) also found that diplontic selection did not exert any great influence on the sector size.

The estimated number of effective initial cells differed considerably from ear to ear in the present investigation. The highest number of 16 cells was estimated for the apical ear following treatment with EMS. The number of effective cells were larger after treatment with chemicals (2 to 16) than with radiations (2 to 11) and more for the apical ear (maximum 16) than for the primary axillary one (maximum 11). The presence of comparatively large number of functional initial cells following treatment with EMS was due to the fact that radiations were more effective in killing the initial cells than chemical mutagens. Osone (1963) reported that the average number of initial cells contributing to the apical ear in rice was five to six. He further observed that the generative tissue of the later formed tillers was derived from a smaller number of initial cells than that of the main stem. The estimated numbers of initial cells for other plants were four for the apical spikes of barley (Gaul, 1961a) and durum wheat (D'Amato et al., 1962), seven to eight for the tassel in maize (Anderson et al., 1949), 10 for Phalaris (Prasad and Godward, 1969) and two for sorghum (Koukis and Reitz, 1955; Sharma, 1965). The smallest size of the mutated sector of an M_1 ear estimated in the present study was 0.06 in treatment with EMS and the number of initial cells was, therefore, calculated as 16. It is evident that this is not an overestimation due to delayed

action of EMS, since as large a number as 11 initial cells have been estimated even after irradiation with gamma rays and fast neutrons, as may be seen from Table XXXIX.

One of the chief limitations in the study of the mutated sector and number of initial cells by analysis of segregation ratios is the availability of small number of plants in a segregating ear-progeny in the M_2 generation. Emphasising this limitation D'Amato (1965) has stated that for the study of mutated sector size, durum wheat producing 35 to 40 seedlings per spike on an average, provides a more suitable material than two rowed barley. Lindgren et al. (1970) have also stated that the problem of measuring the size of the mutated sector in two rowed barley by aid of segregation for chlorophyll mutants is severely affected by the low number of seeds (16 to 20) produced per spike and it will be better to carry out such an analysis with a variety having a large number of seeds per spike. This limitation is further evident from that the highest estimate of the number of initial cells in barley is four (Gaul, 1961a) in spite of the fact that the sector size can be as small as a single anther as reported by Lindgren et al. (1970) and Eriksson and Lindgren (1970), based on the analysis of waxy mutants. The variety of rice producing a mean number of 75 to 100 plants per ear-progeny used in the present investigation has however, provided a more suitable material for the study of mutated sectors than barley or durum wheat. It might be inferred that sectors smaller than 0.06 in size would have been induced in

e present study, but remained undetected without segregating n the M_2 generation. To realise segregation ratios less than 1.3 per cent the number of seedlings in an M_1 ear-progeny must be more than 75. These observations, therefore, indicate that still smaller sectors can be detected and a proper estimation of the number of initial cells made by selecting a variety of rice that produces more than 100 seedlings per ear-progeny.

Deficit and excess of mutants

Mutated genes are expected to segregate in a 3:1 ratio M_3 and later generations giving 25 per cent of mutants in regating populations. But deviations from the normal ratio been found in a large number of M_3 progenies analysed in present investigation. A recessive deficit was more ent than a recessive excess. Deficit of a very high magnitude giving segregation ratios as low as 3.8 per cent (Table XLIII) in the M_3 generation was observed. Deficit of recessive mutants in M_3 and later generations have been reported by Bekendam (1961), Yamaguchi (1962a), Kawai and Sato (1965) and Kawai and Inoshita (1965) in rice, by Koh and Smith (1951) in barley and wheat, by Gaul (1961a, 1964a) in barley, by D'Amato (1961) in wheat, by Blixt (1960) and Gottschalk (1961) in peas and by Beard (1970) in flax. Very low frequencies as obtained in the present study have been reported by Koh and Milan (1956) in barley, by Gottschalk (1961) in peas and by Beard (1970) in flax.

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Based on a comparative study of the M_2 and M_3 segregations, the deficit of recessives in the present investigation could be attributed to various factors such as haplontic elimination of gametes carrying the mutant gene, diplontic elimination of the mutant homozygotes at the zygote or proembryo stage and complex gene interactions governing the mutant phenotype. Deficit of mutants through haplontic elimination, however, was found to occur more frequently than diplontic elimination. Among the 165 ear-progenies, analysed, 29 exhibited haplontic elimination as against 11 with diplontic elimination (Tables XLIII & XLIV). The chief cause of recessive deficit might be thus, the reduced competitive ability of gametes that contain mutations leading to haplontic elimination of male or female gametes as reported by Gaul (1964a) and Moh (1968). Moh and Nilan (1956), D'Amato (1965), Gustafsson (1965), Doll (1968) and Swaminathan (1970) observed that transmission of the mutant gene through the male gamete was less frequent than through the female gamete. Doll (1968) stated that the chloroplast mutant genes in barley affected either the viability of microspores or their ability to take part in fertilisation. Elimination of homozygous mutants through reduced viability of embryos has been reported by Robbelen (1957) in Arabidopsis and Klixt (1965) in peas.

In the present investigation, excess of recessive mutants was observed occasionally in the M_2 generation but very rarely the higher ratios persisted in the M_3 generation. Only two cases of significant recessive surplus were observed

among the 202 mutations studied. They were a *viridis* and a dwarf macro-mutant induced by EMS and NMU respectively (Table XLVII). Kawai and Sato (1965) and Kawai and Inoshita (1965) reported in rice an excess of recessives in the M_3 generation. Gottschalk (1961) found two cases of recessive surplus among the 300 genes studied in peas. The excess of recessive mutants in the M_3 generation recorded in this investigation could have been caused either by the simultaneous occurrence of factor and chromosome mutations (Moh and Smith, 1952; Moh *et al*., 1955) or by selective fertilisation (Gottschalk, 1961).

V. Micro-mutations

The induction of mutations in polygenes controlling quantitative characters can be detected by the estimation of mean and variance of successive generations of mutagen treated populations. It was observed in the present investigation that neither gamma rays nor EMS at the doses employed altered the means of populations with respect to characters such as duration, height of plants, length of ear and number of spikelets. The mean number of ears per plant increased slightly following treatment with either of the mutagens. The increase was most conspicuous in the M_2 generation and the means reverted to that of the control in later generations. Several investigators have reported that the mean values in respect of various quantitative characters were not significantly altered following treatment with radiations

as well as chemical mutagens in rice (Oka et al., 1958; Matsuo and Onozawa, 1961; Ota et al., 1962; Kawai, 1962; Yamaguchi, 1964; Miah and Bhatti, 1968; Sharma and Saini, 1970), in barley (Gupta, 1970), in wheat (Dahl et al., 1968) and in Arabidopsis (Daly, 1960; Bhatia and Van Der Veen, 1965). An increase in the mean number of tillers in rice was reported by Singh (1970).

Variability on the other hand, was found to increase considerably for all characters in treatments with gamma rays as well as EMS in the present investigation. Increase in variance following mutagenic treatments was a common feature observed in quantitative characters by several investigators working on rice (Oka et al., 1958; Bateman, 1959; Kao et al., 1960; Matsuo and Onozawa, 1961; Kawai, 1962; Chari, 1963; Matsuo et al., 1964; Sakai and Suzuki, 1964; Yamagata, 1964; Yamaguchi, 1964; Miah and Yamaguchi, 1965; Miah and Bhatti, 1968; Sharma and Saini, 1970). Increase in variability could be explained to be due to mutations of polygenes governing the quantitative characters. Variability was found to increase with increasing doses of mutagens in the present investigation; the increase was progressive but not linear. Genetic variability was reported to increase in barley with increasing doses of X-rays (Gaul, 1965, 1967) and gamma rays (Gupta, 1970) and in Arabidopsis with increasing concentrations of EMS (Bhatia and Van Der Veen, 1965). Yamaguchi (1964) in rice and Scossiroli (1965, 1970) in wheat had reported that the relationship between increase in dose and variance was not linear.

The magnitude of variability was greater with gamma rays than with EMS at the level of the doses employed in the present investigation in respect of all characters studied in every generation. At these dose levels gamma rays and EMS, however, induced almost similar degrees of reduction in survival and seedling height in the M_1 generation, thereby indicating that gamma rays were more efficient than EMS in inducing micro-mutations in rice. At the same dose levels gamma rays induced higher frequencies of viable and total mutations than EMS, whereas EMS induced higher frequencies of chlorophyll mutations than gamma rays. These results, therefore, indicated that the frequency of induced micro--mutations following mutagenic treatments as reflected by an increase in variability was correlated with the frequency of viable mutations than with that of chlorophyll mutations. Matsuo and Onozawa (1961) reported that the increase in variance in rice was more in the case of radiations than with diepoxy butane and Brock (1965) concluded that ionizing radiations were as effective as chemical mutagens for inducing variability. However, in barley chemical mutagens such as EMS were found to induce more variability than X-rays and gamma rays (Gaul, 1967; Gaul and Aastveit, 1966; Gaul et al., 1966; Gupta, 1970).

Different characters studied in the present investigation were found to respond differently to the induced variation. The magnitude of variability was the highest for height of plants, the induced variability being more

than twice that of the untreated populations at the highest dose of gamma rays (208% in the M_3). The other characters which could be arranged in order of decreasing variance were the length of ear, number of ears, duration and number of spikelets. Oka et al. (1958) found that for a constant dose of radiation the induced genetic variance was more for height of plants than for heading date. Gonzalez and Frey (1965) reported that the magnitude of genetic variability induced was influenced by the character and the genotype treated. Gaul (1961b) stated that the larger the number of genes involved in a character the higher the probability of obtaining an alteration by a mutation of one of the multiple genes concerned.

Gaul (1964a) stated that the radiation-induced variance could be determined as early as in the M_2 generation. In the present investigation, the magnitude of variability was found to differ between generations for the same character. Maximum variability was recorded for the number of ears, length of ear and number of spikelets in the M_2 generation, for height of plants in the M_2 and M_3 generations and for duration in the M_4 generation. Such differences in the expression of variability in different generations for the same character have been reported by Borojevic and Borojevic (1968) in wheat. Efficient use of induced variability would be possible, if the generation in which maximum variability is likely to be released is known.

Though all available data are consistent with the concept that the variance is enlarged, there are considerable differences in the reported results concerning the frequency of occurrence of mutations with both positive and negative effects. The increase in variance in the present investigation was symmetrical for all characters, except the number of ears per plant for which the distribution was skewed on the positive side (Figure 46-1). The symmetrical increase of variability without significant difference in the mean values made Oka et al. (1958), Matsuo and Onozawa (1961), Yamaguchi (1964) and Sharma and Saini (1970) suggest that mutations with positive and negative effects occurred with approximately equal frequency. Gonzalez and Frey (1965) found that variability was shifted in either direction and Gregory (1965, 1968) stated that the distribution of quantitative variability was symmetrical. However, Goud (1967b) in bread wheat, Bhatia and Van Der Veen (1965) and Lawrence (1965) in Arabidopsis and Brock (1970) in subterranean clover observed that the variability was more in the positive direction indicating either a large number of mutations or a high degree of individual effect of mutations in the positive direction.

Various hypotheses have been offered to explain the direction in which the means of populations are altered and the nature and magnitude of variability induced following mutagenic treatments. Gregory (1965, 1966) postulated that the number of negative and positive mutations in the polygene

system is nearly equal and that it is the magnitude of the phenotypic effect of a mutation which gives the negative effects and not its unidirectional character. Brock (1965, 1967) proposed a general hypothesis for the behaviour of induced mutations in quantitatively inherited variation. According to this hypothesis, the theoretical expectation of inducing random mutations in a quantitatively inherited character in a self fertilising organism will depend on the total number of genes involved, the relative proportion of genes with positive and negative effects and the degree to which the genes of the parental genome operate as a balanced set. Further, in species which have previously been subjected to breeding and selection, random mutation results in an increase in the variance and a shift in the mean away from the direction of previous selection. The shift in the mean of an unselected character depends not only upon the previous selection history of the population but also upon whether it is genetically correlated with the character under selection. Swaminathan (1966d, 1969a) working with wheat and barley reported results in support of this hypothesis. Gaul and Aastveit (1966) formulated a different hypothesis stating that by random mutation a change in the mean value of any quantitative character could be expected in a direction associated with reduced vitality and the alteration of the mean was largely independent of the genotype used for the mutagenic treatment. Scossiroli (1965, 1966, 1970) also interpreted the decreasing effect on means as due to detrimental mutations which occurred more frequently than favourable ones. This decreasing effect

on the means would get mitigated by the natural elimination of detrimental mutations in subsequent generations.

The symmetrical increase in variability without alteration of the mean for the quantitative characters now investigated, indicated that mutations with positive and negative effects occurred with approximately equal frequencies. As judged from the magnitude of induced variation, micro-mutations governing plant height were frequently induced in relation to other traits.

VI. Radiosensitivity of varieties

The assessment of radiosensitivity in higher plants has been mostly done using different biological criteria such as germination, survival, plant growth, fertility, chromosome aberrations, chlorophyll deficient chimeras and mutation frequency. The 20 varieties of rice belonging to the subspecies indica and japonica of Oryza sativa studied in the present investigation exhibited marked differences in sensitivity as expressed by differences in lethality, injury and sterility induced in the M_1 generation by X-irradiation. The range for sterility was the highest (23 to 52%) and lethality the lowest (2 to 16%) among the varieties (Table LVI). Thus, the magnitude of differences in sensitivity between varieties depended on the criteria adopted to measure the radiation effects. The varieties were also found to yield chlorophyll mutations in different frequencies in the M_2 generation (Figure 48). Intervarietal differences in

radiosensitivity were recorded in rice by Yamaguchi (1956), Matsuo et al. (1958), Fujii (1962), Bilquez et al. (1965), Myttenaere et al. (1965), Ukai (1967), Mikaelsen and Navaratna (1968), Miah et al. (1970) and Singh (1970). Differences in radiosensitivity between varieties were also reported in barley (Matsumura and Fujii 1957; Milan, 1964; Mikaelsen and Brunner, 1968), in bread wheat (Gaul and Aastveit, 1966; Goud, 1967a; Borojevic and Borojevic, 1968; Varughese and Swaminathan, 1968), in durum wheat (Scarascia et al., 1961; D'Amato., 1962; Donini et al., 1964) and in ragi (Kumar et al., 1970).

Among the varieties now studied, those belonging to subspecies indica were found to differ in radiosensitivity from those belonging to subspecies japonica. Intervarietal differences within a subspecies were generally less than the differences observed between subspecies. Many investigators have stressed the higher radiosensitivity of japonica varieties than indicas (Fujii, 1962; Kawai, 1962, 1963a; Yamaguchi, 1964; Swaminathan et al., 1970; Singh, 1970) Differences in radiosensitivity of subspecies were also reported in Triticum aestivum by Swaminathan (1965) and Rana and Swaminathan (1967). It was evident from the present studies that varieties belonging to subspecies indica were more resistant to X-rays when judged on the basis of lethality and injury, but were more sensitive when evaluated on the basis of sterility and chlorophyll mutation frequency (Table LVI) than the varieties belonging to

subspecies *japonica*. Such a difference based on the criteria of estimation of sensitivity was markedly exhibited by varieties of the tropical *japonica* group such as Tainan-3 and Taichung-65. Contrary to *indicas* these varieties were highly sensitive based on lethality and injury but highly resistant based on sterility and mutation frequency. Siddiq and Swaminathan (1968a) also found that Taichung-65 was highly sensitive but yielded only a low mutation frequency.

An examination of the morphological features of the varieties studied and their relationship to radiosensitivity, revealed that an association exists between the length of grain and sensitivity. With an increase in length of grains, the M_1 sterility and M_2 chlorophyll mutant frequency increased, whereas M_1 lethality showed an inverse relationship. Mikaelsen and Navaratna (1968) found that the varieties with the smallest grain were the most radioresistant. However, Maraheri (1969) did not find any such association between sensitivity and characters such as duration, grain size and shape. The inter-varietal differences in radiosensitivity in rice was reported to be due to differences in genotype (Matsuo *et al*., 1958; Chao and Chai, 1961; Fujii, 1962; Miah and Bhatti, 1968 and Maraheri, 1970a), in cytoplasm (Yamaguchi and Kobayashi, 1960; Yamaguchi, 1964), in chemical composition (Mytteneare *et al*., 1965) and in endogenous levels of auxin and ascorbic acid (Goud *et al*., 1967). Differences in sensitivity of varieties even within the subspecies *indica* being thus well expressed,

it is imperative that in programmes of mutation breeding an initial assessment of the efficiency of the mutagens with reference to the varieties should be undertaken.

SUMMARY

SUMMARY

Studies were undertaken to obtain precise information on the efficiency of radiations (gamma rays, X-rays and fast neutrons) and chemical mutagens (EMS, NMU, DES, MMS and MNNG) in inducing mutations in rice using a single variety of Oryza sativa subsp. indica. Problems relating to the enhancement of the efficiency in induction of mutations by regulating the mutagen treatment as well as by pretreating the biological material were investigated. Factors such as the organisation of the embryo and the number of primordial cells involved in the origin of each panicle which influenced the recovery of mutants were determined. The possibility of inducing micro-mutations in quantitative characters was also explored. Differences in sensitivity of 20 varieties of rice belonging to the subspecies indica and japonica of Oryza sativa to radiation treatments were estimated.

2. Germination of rice seeds was not affected by radiations even at very high doses. Chemical mutagens inhibited germination and the reduction in percentage was progressive with increasing doses. Radiation induced lethality was manifested subsequent to germination through a prolonged lethal action even at the advanced stages of plant growth, whereas lethality induced by chemical mutagens was imminently expressed mostly through inhibition of germination. In chemical mutagen treatments, M_1 seedlings which could reach the fourth leaf stage survived to maturity.

3. Mutagens induced a greater inhibitory effect on the root than on the shoot. Gamma rays and NMU inhibited the growth of plants more effectively than the other mutagens. Following irradiation with fast neutrons the plants showed less variability in height than after treatment with X-rays and gamma rays. The M_1 plants recovered from injury as growth proceeded.

4. Chemical mutagens, when compared with radiations had induced low degrees of sterility. The decrease in fertility following irradiation was drastic up to the middle doses with a saturation effect at high doses. Fast neutrons induced more sterility than sparsely ionising radiations per unit dose.

5. The three main estimates of the effects of mutagens in the M_1 generation viz., lethality, injury and sterility did not show similar trends with increase in doses of mutagens. Radiations induced more of sterility than lethality and injury. NMU induced more of lethality and injury than sterility, whereas EMS induced these effects in almost equal intensities.

6. X-rays and gamma rays induced biological effects with the same intensity, whereas fast neutrons were several times more effective than the sparsely ionising radiations irrespective of the criteria employed for the assessment of relative biological effectiveness.

7. Chlorophyll deficient sectors on M_1 plants were induced frequently by chemical mutagens such as NMU and EMS.

In this respect NMU was more effective than EMS. However, the frequency of M_1 chlorophyll chimeras did not prove to be a reliable criterion for comparison of the effects of different mutagens. Chlorophyll deficient sectors on leaves and panicles were of independent origin. It would be possible to realise a high frequency of chlorophyll mutations in the M_2 generation by selecting M_1 panicles possessing chlorophyll deficient sectors.

8. The mutation frequency estimated as number of mutations per 100 M_1 spikes was as efficient as that estimated as the number of mutants per 100 M_2 plants, when the study was confined to the preformed ears. The dose-frequency relationship remained the same irrespective of the method of estimation of mutation frequencies. The frequencies decreased at high doses even when estimated on M_2 plant basis.

9. Gamma rays, fast neutrons, EMS and NMU had induced high frequencies of chlorophyll mutations. In terms of maximum frequencies induced, gamma rays were superior to fast neutrons and EMS was superior to NMU. But per unit dose fast neutrons were more effective than gamma rays and at equimolar concentrations NMU was more effective than EMS. In terms of highest frequencies as well as at LD_{20} for injury, EMS induced the maximum frequencies of chlorophyll mutations.

10. The mutagens were ranked in decreasing order of potency as gamma rays, EMS, NMU and fast neutrons in terms of the maximum frequency of viable mutations induced, and based on

efficiency at doses inducing similar biological effects (LD_{20} for injury) they were in the order gamma rays, EMS, fast neutrons and NMU. Thus, in terms of either criterion, gamma rays were the best in inducing viable mutations.

11. As per the estimates of mutagenic "effectiveness" and "efficiency", fast neutrons were the most effective and efficient among the radiations. Among the chemical mutagens, NMU was the most effective but EMS was the most efficient.

12. The frequency of chlorophyll mutations increased with decreasing seed fertility following treatment with gamma rays as well as EMS, when M_1 ears were selected at random. However, at the lowest fertility class the mutation frequency decreased. This reduction is attributed to the elimination of mutants.

13. The spectra of induced chlorophyll and viable mutants differed between radiations and chemical mutagens. Albina was the most frequent type following irradiation. With chemical mutagen treatments there was a decrease of albina followed by an increase in viridis. Erectoides and other macro-mutants were frequent following treatment with fast neutrons and NMU. Mutants affecting culm length were common among the types induced by EMS and mutants with altered duration and grain size were more commonly induced than others by gamma rays.

14. A large number of viable mutants was isolated following treatment with different mutagens. Most of them

were monogenic recessives appearing in the M_2 generation and breeding true in later generations. A few double recessive mutants and one with incomplete dominance were spotted out. The origin of the viable striata mutants had been traced to plastid mutations.

15. Most of the induced viable mutants were macro--mutants in which a desirable change in one character was associated with an undesirable effect in another character. Mutants with changes in individual characters such as the height of plants, duration and size of grains were also isolated. Changes in height of plants were mostly towards dwarfs and semi-dwarfs and those in maturity period were frequently towards late types. The 421 viable mutants isolated in the single variety represented variability for almost every character of the rice plant, indicating the scope for altering any character in rice through induced mutations. The mutants constituted a substantial assemblage in the subspecies *indica* of *Oryza sativa*.

16. In the combination treatments of radiations and MMU the mutation frequencies estimated as number of mutations per 100 M_1 spikes, were not higher than the values expected on the basis of additive effects. When estimated as number of mutants per 100 M_2 plants the frequencies revealed more than additive effects. The synergistic effects on mutant frequencies were shown to be due to an increase in the segregation ratio of mutants. This effect was most pronounced at the higher dose combinations of fast neutrons and MMU.

17. Recurrent treatment with gamma rays and alternate treatment with gamma rays and NMU neither increase the sensitivity of rice seeds nor enhance the frequency of induced mutations thereby indicating that rice, in spite of its secondary polyploid nature behaved like a diploid with respect to its response to the action of mutagens.

18. Sensitivity to NMU increased with increasing periods of presoaking in water and reached a maximum at the 32 hour duration of presoaking. There was a decrease in sensitivity when presoaking was extended beyond 32 hours. The specificity of the sensitivity peak in relation to the period of presoaking was significant. The first DNA synthesis taking place in the cell initials had been reported as the most important factor responsible for the peak in sensitivity at the 32 hour presoaking period.

19. The frequency of chlorophyll mutations increased with an increase in presoaking time reaching a maximum at the 40 hour period. The conspicuous increases were during the periods of 16 to 20 hours and 24 to 28 hours. The enhanced efficiency during the 16 to 28 hour period of presoaking was attributed to the synchronisation of treatment time with the S phase of DNA synthesis. The relative percentages of different types of chlorophyll mutants induced by NMU were altered by presoaking of seeds for varios periods. Xanthas and tigrinas appeared in low frequencies only in seed lots presoaked for 20 hours and beyond. The delayed development of xanthas and

tigrinas was the most conspicuous feature of the chlorophyll mutant spectrum.

20. The estimated size of the mutated sector and number of initial cells were found to differ from ear to ear. The sectors on the apical ears were smaller than those on the primary axillary ears. The number of initial cells was found to be more with a maximum of 16 for the apical ear than that for the primary axillary ear having a maximum of 11. The sectors induced by chemical mutagens were smaller in size than those induced by radiations. The number of effective initial cells was larger after treatment with chemical mutagens (2 to 16) than with radiations (2 to 11).

21. The smallest size of the mutated sector estimated was 0.06 in treatment with EMS and the number of initial cells was, therefore, 16. This could not be considered as an over--estimation due to delayed action of EMS, since as large a number as 11 initial cells was estimated after irradiation with gamma rays. The variety of rice used in this investigation producing a mean number of 75 to 100 plants per ear-progeny provided a suitable material for the study of mutated sector size and estimation of the number of initial cells.

22. Deviations from normal segregation ratios were found in the M_3 generation. A deficit of recessive mutants was more frequent than an excess. Deficit through haplontic elimination of gametes was more common than that through diplontic elimination. Excess of recessive mutants was

occasional in the M_2 generation but the higher frequencies seldom persisted in the M_3 generation.

23. Neither gamma rays nor EMS induced alterations in the means of populations in respect of characters such as duration, height of plants, length of ear and number of spikelets. The mean number of ears per plant increased slightly following treatment with either of the mutagens.

24. Variability increased considerably in treatments with gamma rays as well as EMS. The magnitude of induced variability was greater with gamma rays than with EMS in respect of all characters in every generation. Gamma rays were thus, more efficient in inducing micro-mutations than EMS. Variability was the highest for height of plants. The magnitude of induced variability was found to differ in different generations. The increase in variance was symmetrical for all characters except for the number of ears per plant, for which the distribution was skewed on the positive side. The symmetrical increase in variability without alteration of the mean had indicated that mutations with positive and negative effects occurred with approximately equal frequencies.

25. The 20 varieties of Oryza sativa exhibited marked differences in sensitivity to X-rays as expressed in terms of lethality, injury and sterility in the M_1 generation and chlorophyll mutation frequencies in the M_2 generation.

Varieties belonging to subspecies *indica* differed in sensitivity from those belonging to subspecies *japonica*. The *indica* varieties were resistant to X-rays when judged on the basis of lethality and injury, but were highly sensitive when evaluated on the basis of sterility and chlorophyll mutation frequency. Differences in sensitivity of varieties to mutagens within the subspecies *indica* had become increasingly clear.

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