INDUCTION OF GENETIC VARIABILITY IN GUINEA GRASS (*Panicum maximum* Jacq.) Var. Makuenii

By

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THESIS

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VELLAYANI, TRIVANDRUM

1989

DECLARATION

I hereby declare that this thesis entitled "Induction of genetic variability in guinea grass (*Panicum maximum* Jacq.) var. Makueni" is a bonafide record of the research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "Induction of genetic variability in guinea grass (*Panicum maximum* Jacq.) var. Makueníi" is a record of research work done independently by Smt. Rani.N under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Guinea grass is the most popular fodder grass in Kerala and has become well acclimatised in this region. Drought resistance and hardy nature of this plant have facilitated its cultivation as a rainfed crop under partial shade conditions of coconut gardens in Kerala. But, however, under Kerala conditions, the green fodder yielding ability of this grass in partial shade of coconut gardens has been found not up to the mark especially in summer months.

Panicum maximum is a facultative apomict with less than five percent cross fertilization. The apomictic reproduction and vegetative propagation prevalent in this crop have imposed serious limitations in genetic improvements by conventional methods of breeding. The evaluation of available clones at the College of Agriculture, Vellayani, revealed that the genetic variability in the crop is very limited (Pillai et al., 1974). Gross incompatibility between species also restricts the attempts for interspecific crosses as indicated by the results of the studies on interspecific hybridisation between P. maximum. and P. repens. In these circumstances, special techniques such as induction of mutations, have become more relevant.

At the turn of the century, the enormous potential usefulness of induced mutations, for plant breeding has stirred the imagination of many geneticists. The scope for producing better species of cultivated plants and domesticated animals by mutation techniques was

anticipated by them. In contrast to the investigative studies during early periods, which were of purely experimental nature, the seventies and eighties witnessed the practical utilisation of induced mutations on a wide range of crops. Induction of mutation by radiation and chemicals has become a quite useful tool in modern plant breeding.

The dual method of propagation, viz., seed and slip propagation prevalent in guinea grass makes it an excellent material for genetic improvement through mutations. Identification of superior variants followed by vegetative multiplication can lead to immediate improvement in the level of fodder production since seed propagation need not be resorted to for multiplication. The genetic variation induced can directly be carried forward without deterioration through slip propagation.

The natural population of such grasses which are descendants of sexual and asexual reproduction and which are at present propagated through vegetative means, are expected to be highly heterozygous. The vegetative propagation helps to preserve the heterozygosity. The heterozygosity makes possible the detection of induced mutations in the M_1 generations itself. Most mutations being reccessive can be detected easily in the M_1 generation. Vegetative propagation of such M_1 plants into M_1V_1 clonal progeny and a critical evaluation

of their performance compared to their clonal progeny can be considered as an efficient method to isolate superior clones (Nair, 1979).

In present investigation an attempt has been made to induce variability in the cultivar Mukuenii of P. maximum employing gamma radiation and Ethyl methane sulphonate and the results are tabulated and discussed.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I Origin and History

Guinea grass of genus Panicum belongs to the tribe Paniceae. Guinea grass (Panicum maximum J.) is a fodder grass, native of Tropical Africa and was introduced to India in 1793 (Bor, 1960). The major fodder grasses coming under this genus are P. antidotale, P.humilis, P. odoratum and P. stapfianum. Guinea grass is a favourite fodder among all types of livestock and has become the most popular fodder grass of Kerala. The hardy nature and drought resistance of the crop is congenial for growing as an intercrop in coconut gardens (Prasad and Gopimony, 1982). Early results of the evaluation of over 200 genotypes distinguished 38 cultivars and ecotypes of grass species as being particularly valuable especially P. maximum 'Mukuenii' which was resistant to Tilleria species. Makuenii was introduced into Australia as CPI 37990 from Kenya. Makuenii is a high yielder and notably aggressive and persistant (Machado and Munoz, 1982).

a) Description of Species

Guinea grass is an erect, dense, variable, perennial grass growing up to 3m high, forming dense tufts up to 1 to 2m diameter. Leaves are basal, linear, greatly elongated, alternate and in two ranks on the stem, the basal portion forming a sheath enclosing the

stem but margins not coalescent. A membraneous ligule is usually present or represented by a row of hairs at the point of joining of the sheath and the blade (Prasad and Gopimony, 1982). Makuenii is hirsute with wider leaves than other Guinea grass and the canopy is less erect. Annual total dry matter yields of Mahuenii are similar to those of other commercial varieties. In temperate regions although it makes less summer growth, it substantially out yields other varieties in cool season (Anon, 1974). In P. maximum different clones show variation in growth habit, tillering, waxiness and hairiness of plant parts, size of stem, leaves and panicle and leaf stem ratio, plant height, tiller number and yield of green matter per clump. Population differentiation has produced two ecotypes, one characterized by late flowering, late tillering and tall plant types and another characterized by late flowering late tillering and short plant type (Usberti and Jain, 1970). In Panicum maximum two patterns of panicle production were noted. In some accessions there was a pronounced peak of panicle production followed by a sharp decline while in others the panicles were produced at a low but a steady rate over an extended flowering period (Javier, 1970). Inflorescence is a loose panicle with biflowered spikelets. The upper florets are hermaphrodite (Purseglove, 1975).

b) Cytology

Jauhar and Joshi (1966) reported the chromosome number of P. maximum as 2n=32. They described the species as an

'interspecific autoploid. Varying numbers of quadrivalents, trivalents, bivalents and univalents were observed during meiosis. The chromosome number 2n=32 seems to have been derived through a multiplication of basic number x=9 chromosomes, followed by elemination of four chromosomes during course of evolution, rather than the multiplication of basic number x=8. Chen and Hsu (1961) reported the n count of P. maximum as n=16. Purseglove (1975) described its chromosome number as 2n=18,36,48. Variants with 2n=36 was inferior in forage production than the normal 2n=32 clones (Ramaswamy and Raman, 1971).

c) Mode of reproduction

Brown and Emery (1958) concluded this as a highly apomictic crop species. Comparison of selfed and open pollinated progenies showed wide variability in selfed progenies (Raman, 1971). Hybrids between small sexual strains and late maturing giant strains, showed that recombination of desired characters can be obtained in a breeding programme. However cross incompatibility between species restricts the attempt for interspecific crosses as indicated by results of studies on interspecific hybridization. Sexual tetraploids are obtained by colchicine treatment of diploids (Pernes and Rene Chaume, 1973).

Henna et al. (1973) in P. maximum Jacq. found that inheritence data based upon Progeny of selfed sexual plants and sexual apomictic hybrid indicated that sexuality was dominant. The sexuality was found

to be controlled by at least two or more dominant alleles and most accessions set seeds when plants were selfed (Burton et al., 1973). Sexual plants hybridize readily with apomictic pollinators to give progenies that seggregate for sexual and apomictic modes of reproduction. Ideally an apomictic breeding programme requires both cross-compatible sexual plants and obligate apomictic plants which when hybridised produce true breeding apomictic progeny (Bashaw, 1974). Many scientists have reported the species as a facultative apomict with less than 5% cross fertilisation (Jauhar and Joshi, 1966; Purseglove, 1975). Sexual germplasm of P. maximum normally a facultative apomict has now been produced.

A comparison of selfed and open pollinated progenies showed wide variability in selfed progenies (Ramaswamy and Raman, 1971). Burton (1978) recommended strategies for improving forage grasses using some of suggested approaches such as hybridization, use of self incompatibility or cytoplasmic male sterility to obtain F_1 hybrid varieties. Within population variability was greater on the average on sexual group, but in a few cases heterogeneity was greater on 'asexual' groups. Savidan (1980) in P. maximum made crosses between sexual, pistillate and 8 apomictic staminate parents and got dihaploids (2n=16) thought to have arisen by parthenogenesis, hexaploids (2n=48) from fertilization of unreduced egg cells and octoploids (2n=64).

II Induction of variability

Genetic diversity in parent population is the prime necessity for any crop improvement programme. The importance of artificially

induced mutation in cultivated plants for inducing variability has been amply demonstrated by relatively large number of mutant varieties released all over the world. It was De Vries in 1901 who first induced mutation and variability. The use of X-rays for inducing mutations was suggested by De Vries in 1904 (c.f. Blakeslee, 1936), Koernicke in 1905 and Gager in 1908. However the conclusive proof that ionising radiations induce mutations was presented by Muller (1927) in Drosophila and Stadler (1928), Gager and Blakeslee ((1927) and Goodspeed (1927) in plants. These early works did not contribute much to plant improvement. The seventies witnessed the practical utilisation of induced mutations in a wide range of crops (Gregory, 1972).

The search for chemicals capable of causing mutations began even before the discovery of mutagenic effect of X-rays (Auerbach, 1967). Schiemann (1912) attempted chemical mutagenesis early in century. Auerbach and Robson (1942, 1947) in England and Rapoport (1948) in U.S.S.R demonstrated the mutagenesity of mustard gas. Since then a number of chemicals possessing mutagenic properties have been identified and their effects studied.

Alkylating agents are the most efficient in a wide array of organisms among the numerous radio-mimetic chemicals now known (Auerbach, 1961). Within the alkylating group monofunctional agents in general and ethylmethane sulphonate in particular appear to be more efficient in several organisms including higher plants

(Swaminathan et al., 1962). The mutagenic efficiency of ethyl methane sulphonate was first demonstrated by Heslot et al. (1959) and later by Ehrenberg (1960). Of the various chemical mutagens ethylmethane sulphonate was reported to possess properties favouring high mutagenic effectiveness as well as high mutagenic efficiency.

A wide range of both physical and chemical mutagens is now available. The relative advantages and disadvantages of the different mutagens have been investigated by many workers. Ehrenberg et al. (1961) and Heiner et al. (1960) showed that some chemical induced mutations with frequencies two to three times higher than the highest frequencies obtained following radiation treatments. Sato (1966) reported EI and EMS to be more powerful mutagens than radiations in inducing visible mutations in rice. Physical mutagens like X-rays, fast neutrons and gamma rays have been frequently employed in inducing useful mutations in crop plants as compared to chemical mutagens (Swaminathan 1969b). The available literature on mutation breeding is flooded with works in induced mutations through ionising radiation. In sexually propagated crops chemical mutagens yield very high mutagen frequencies and in most cases they were more efficient than ionising radiations (Kamra and Brunner, 1970). However, it is premature to assess the merits of chemical mutagens on the basis of a number of varieties to which they have given rise, since extensive work with chemical mutagens have begun

only in 1960 following the introduction of EMS. As such, the choice between the chemical and physical mutagens for induced variability is only arbitrary. Swaminathan (1969c) however, opined that neutrons among radiations and EMS among chemicals were generally the mutagens of choice. Nair (1971) ranked the mutagens as gamma rays, EMS, NMU and fast neutrons in terms of frequency of viable mutations they induced. Based on their efficiency at doses inducing similar biological effect they were reported to be in the order gamma rays, EMS, fast neutrons and NMU. He thus, concluded that gamma rays were the best in inducing viable mutations. Yamashita et al. (1972) concluded that chemical mutagens produced 5-8 times higher mutations than gamma radiations. Singh et al. (1978) showed that EMS caused more biological damages than gamma rays. Prasad and Gopimony (1982) induced viable mutations in guinea grass using EMS and gamma rays. Vijayagopal and Nair (1985) induced several useful mutants in rice using EMS and gamma rays.

Since the premier works of Yamada (1917) and Nakamura (1918) which indicated increased yield with exposure of seeds to X-rays for short periods, the literature on induced mutations accumulated tremendously. The progress in mutation research and practical achievements in crop improvements have been reviewed by many investigators (Auerbach, 1961,1967; Gaul, 1961, 1964; Sparrow, 1961; Sparrow et al., 1965, 1969; Gustafsson, 1963, 1969; Swaminathan, 1969a and b; Nair, 1971; Nilen et al., 1965; Hajra, 1979; Mahadevappa

et al., 1981, Lal and Richharia, 1982; Ganshan and Whittington, 1983). Although the mutation studies were very common in cereals like wheat, barley, rice, oats and sorghum such studies are very rare in forage grasses. This review therefore gives greater emphasis on the works done in cultivated members of Graminae family in general and Panicum species in particular.

III Effect of mutagens in the M_1 generation

Mutagen treatments produce genetic and physiological effects, both desirable and undesirable in biological systems. While lower doses may not reveal any severe effects, the higher doses will produce gross visible disturbances. The mutagenic sensitivity of plants is usually measured by parameters such as germination, survival, seedling injury, plant growth, fertility, chlorophyll deficient chimera etc.

1. Germination

Matsumura and Mubuchi (1964) observed decreasing germination with increasing dosages of radiation in several crop plants. However, germination was not much affected by radiation, though damage occurred after sometime (Goud et al., 1967; siddiq, 1967 and Ganashan 1970). Both delayed germination and reduction in germination percentage were noted at higher doses of gamma rays (Shirshow and Shain, 1966; Nair, 1971, Vijayagopal and Nair 1985). Delay in germination was found to increase with levels of the mutagen in case of X-rays (Athwal 1963). Similar results were presented by Maslov and Stepanova (1967) and many other workers using gamma rays. Several other workers reported decrease in germination percentage after irradiation treatments (L'vova

and Konoravskaya, 1974; Polomino, 1979 in barley; Reddy and Smith, 1975 in sorghum, Vaidyanathan, 1973 in Panicum antidotale; Valeva 1976 in wheat and Ayyamperumal, 1977 in ragi). Sinha and Godward (1972) observed a reduction in germination using gamma rays due to the disturbance caused at the physiochemical level of the cells or due to acute chromosomal damage or both.

On the other hand chemical mutagens greatly reduce germination of seeds ((Rao and Ayengar, 1964; Siddiq and Swaminathan 1968; Sri Ramulu, 1969; Nair 1971). The inhibition of germination due to EMS was thought to be due to the formation of acids during hydrolysis and change in pH of medium making it toxic (Freese, 1963). Severe reduction in germination was reported by Raveendran (1976) and Ayyamperumal (1977) in ragi; and Choudhary (1978) in wheat. Prasad and Gopimony (1982) found reduced germination of guinea grass seeds at higher doses of EMS.

Contrary to this Rao and Natarajan (1965) in barley; Siddiq and Swaminathan (1968) in rice and Goud et al. (1970) in Sorghum reported that low doses of ethylmethane sulphonate had little or no effect on germination. Raman and Soriano (1972) and Vereshchagin (1974) in rice and Vaidhyanathan (1973) in Panicum antidotale reported that EMS treatment had little effect on germination. Increase in percentage of germination was attributed to increased activity of certain enzymes induced in synthesis of auxins (Casarett, 1968).

2. Survival of Seedlings

The number of seedlings surviving after mutagen treatment has been found to decrease with increasing dose of mutagen both physical and chemical (Siddiq, 1967, Siddiq and Swaminathan, 1968; Ganashan, 1970; Prasad and Gopimony, 1982). Final survival in sorghum populations was found to be affected to a great extent with higher doses of radiations (Goud et al., 1970; Goud, 1972; Ramulu, 1974; Reddy and Smith, 1975). Contradictory reports were presented by Ganusuvichyute (1971) in barely. However several workers reported reduction in survival after gamma radiation in different crop plants. (Singh, 1971 in rice; Ravendran, 1976 in rice; Valeva, 1976 and Chowdhari, 1978 in wheat, Sapra et al, 1978 in Triticale).

Siddiq and Swaminathan (1968) observed little or no effect of low doses of EMS on the survival of rice. Ethyl methane sulphonate was found to reduce survival slightly (Swaminathan, 1970). Rahman and Soriano (1972) also got concurrent results. Vaidhyanathan (1973) in Panicum antidotale noticed no appreciable reduction in the survival of seedlings after treatment with EMS. In contrast to the above observations, Singh et al. (1978) reported reduction in survival as the concentration of the mutagen increased. A comparative study of mutagenous chemicals on survival revealed that EMS caused least lethality (Nair 1971), Vijayagopal and Nair (1985) reported that while

gamma rays induced lethality of seedlings at all doses with drastic effect at high doses, the low doses of EMS were not lethal to the seedlings.

3. Seedling Growth

Seedling growth is measured by the rate of reduction in shoot growth. It has been used as a reliable estimate of damage in several radiobiological studies. Caldecot et al., (1954) observed a reduction in growth of barley seeds after X-ray irradiation. The decrease in growth of seedlings following X-ray irradiation was reported due to destruction of auxin caused by ionising radiation (Smith and Kerstan, 1942). Increase in dose of mutagen progressively reduced the seedling height, (Gottschalk, 1967). Concurrent results were reported by Ramulu (1974) in Sorghum. Ananthaswamy et al. (1971) reported seedling growth inhibition by 50-62% at low doses of irradiation in wheat. Reddy and Smith (1975) found reduction in seedling height in sorghum with increasing doses of gamma rays. Valeva (1976) reported greater susceptibility of wild wheat forms to mutagenic treatments. Sapra et al. (1978) observed no reduction in seedling height upto 10 Krads of gamma radiation and severe reduction at higher doses in Triticale. Javeed Iqbal (1979) recorded large reduction in seedling height in sorghum as doses of irradiation increased. However Prasad and Gopimony (1982) noted a stimulatory effect on seedling height at certain low doses of gamma irradiation.

In rice, Soriano (1968) reported reduction in seedling height consequent on ethyl methane sulphonate treatment. Similar reports were published by Sharma (1970) in barley. Rahman and Soriano (1972) indicated a linear relationship between seedling injury and concentration of ethyl methane sulphonate in rice. In pearl millet, reduction in seedling height was reported by Singh et al. (1978) particularly with ethyl methane sulphonate.

4. Plant height

Reduction of height of plants have been more drastic generally in treatments with radiation than with chemicals (Siddiq, 1967; Singh et al. 1978). With gamma rays a linear relationship between a dose and the reduction in shoot growth has been reported. Matsuo et al. (1958); Yamaguchi (1964) and Masima and Kawai (1958) found that plants were less variable in height after irradiation with neutrons and X-rays. Kapoor and Natarajan (1970) in barley; Walker and Sisodia (1969) in sugarcane; Ayyamperumal (1977) in ragi; Vijendra Das (1978) in bajra and Sree Ramulu (1974) in sorghum reported reduction in plant height due to gamma irradiation. Gamma rays caused significant plant variability in wheat (Khadr and Shukry, 1972). However they reported that the variation was not accompanied by any shift in population mean, and in most cases variation was equally distributed around population mean. Reduction in growth following gamma irradiation was reported by Vaidhyanathan (1973) in Panicum antidotale.

Among chemicals, nitroso methyl urea was found to be highly effective in reducing the height of seedlings (Singh, 1970). He further reported that M_1 seedlings recovered in growth after 45 days. Nair (1971) observed greater inhibitory effect of radiations and most of the mutagenic chemicals on the root in comparison with effect on shoot. He further reported that with higher doses of gamma rays and NMU, the inhibitory effect on root was twice as intense as that on the shoot. Nair (1971) concluded that gamma rays and NMU were more effective in reducing height of plants. In sorghum, Sree Ramulu (1974) and in ragi, Ayyamperumal (1977) reported gradual decrease in mean of plant height as concentration of chemical mutagens increased.

5. Tiller Counts

Many workers have reported decreasing progeny means and variation in tiller number in irradiated population of wheat (Bhatia and Swaminathan, 1962 and Goud 1967). Dhonukshe and Bhowal (1976) concluded that mean tiller number per plant was significantly increased in irradiated populations and observed the shifting towards positive direction only. Variances were also found to increase in all treatments. Listikova and Shcherbako (1976) observed increase in tillering with increase in radiation dose in wheat. But in sorghum Javeed Iqbal (1979) reported a large reduction in mean tillering as dose of radiation increased. Gielo and Starzyeki (1978) observed significant increase in range of variation in tillering in rye following gamma radiation. However

Prasad and Gopimony (1982) did not find any significant effect on the tiller number in the gamma irradiated population at the doses used.

Ethyl methane sulphonate treatment reduced tillering in wheat and barley (Kapoor and Natarajan, 1970). Sokolov and Khvostova (1972) observed reduction in tillering in barley following EMS treatment. Mallick et al. (1979) also reported reduction in mean tiller number in rice. However Ayyamperumal (1977) in ragi did not find statistically significant results in tiller counts. Prasad and Gopimony (1982) also recorded similar observations in P maximum.

6. Pollen Sterility

A mutagenic treatment generally results in reduced fertility. The sterility mostly caused by chromosome abberations can be quantitatively determined by counting sterile pollen or using seed setting. Decreased pollen and seed fertility show a linear relationship with mutagen doses (Beachell, 1957; Chang and Hshish, 1957; Yamaguchi, 1964; Siddiq, 1967; Singh, 1970, Awan and Bari, 1979). Henderson (1963) and Yeh and Henderson (1963) indicated a decrease in fertility with increase in dose upto a certain level beyond which there was however a saturation effect. Henderson (1963); Yeh and Henderson (1963); Yammaguchi (1964) and Siddiq (1967) found that neutrons reduced fertility more severely than X-rays and gamma rays. Decrease in fertility in sorghum following physical mutagen treatment was observed by Goud et al (1970) and Ramulu (1974). Concurrent results were

also reported in rice (Vijayagopal and Nair, 1985). An inverse relationship between grain fertility in M_1 and doses of gamma rays was reported in sorghum (Reddy and Smith, 1975 and in rice Yamaguchi 1976). Prasad and Gopimony (1982) recorded a positive correlation between pollen sterility and increase in doses of gamma rays in P. maximum. Siddiq and Swaminathan (1968) recorded that chemical mutagens induced more sterility compared to radiations. Chemical mutagens such as diethylsulphate (Rao and Ayengar, 1964; Sato, 1966) ethylene oxide (Sato 1966), nitrosomethylurea and ethylmethane sulphonate (Siddiq and Swaminathan, 1968; Nair, 1971) methyl methane sulphonate and methyl nitroso guanidine (Nair, 1971) were reported to induce much less sterility than radiations. Increased doses of ethyl methane sulphonate reduced fertility of pollen in guinea grass (Prasad and Gopimony, 1982).

7. Chlorophyll chimeras:-

Incidence of chlorophyll deficient sectors on the leaves of M_1 generation of plants in cereals after mutagenic treatments has been recorded by several workers. Chlorophyll disorganisation was reported to be one of many effects of radiation (Gustaffson, 1947). Shastri and Ramiah (1961) and Siddiq (1967) observed chlorophyll deficient sectors on the M_1 plants of rice. Tanaka (1970) recorded such sectors in the haploid plants following chronic gamma irradiation. Nair (1971) obtained plants with chlorotic streaks after treatment with fast neutrons at

a very low frequency, but not on treatments with X-rays or gamma rays. He further recorded that the plants with chlorophyll dificient sectors did not show a clear dependence on dose in rice. Plants with chlorophyll deficiencies were observed following treatment with chemical mutagen such as ethyl methane sulphonate and nitrosomethylurea by Siddiq (1967), Singh (1970) and Nair (1971). The frequency of plants with chlorophyll deficient sectors was found to increase progressively with increasing doses of chemical mutagens (Nair, 1971). Prasad and Gopimony (1982) recorded chlorophyll chimeras, in P. maximum after treatment with gamma rays and EMS. They also reported an increase in frequency of chlorophyll chimeras with increasing doses of mutagens.

8. Morphological abnormalities

Plants with drastic changes are eliminated due to rigorous diplontic selection and naturally surviving plants have less abnormalities (Swaminathan, 1970 and Goud et al., 1970). Gunckal and Sparrow (1961) and Sax (1963) obtained beneficial effects such as increase in strength and leaf thickness using low doses of irradiations. Goud et al. (1970) observed that small grains, sterile ears, lax ears, dwarf, tall and early type of abnormalities were less in higher doses. Kapoor and Natarajan (1970) reported narrow and thick leaves, shorter ears and late flowering in barley after irradiation. Strong stemmed and erectoid ear mutants were reported in barley with gamma rays (Filev, 1972).

Vaidyanathan (1973) in P. antidotale found mosaic pattern of leaves and other leaf abnormalities after gamma ray treatment. Dwarf mutant and plants with narrow leaves were recorded by Prasad and Gopimony (1982) in P. maximum after treatment with gamma ray and EMS. They observed leaf width variations in EMS treated plants. Vijayagopal and Nair (1985) recorded several morphological abnormalities in mutagen treated rice plants.

IV. Study of M_1 V_1 clonal progeny

For various reasons, vegetatively propagated crops form a suitable group for application of mutation breeding technique. Very often mutations are the only source of variability in sterile plants or on obligate apomicts.

Many workers have reported favourable mutations on vegetatively propagated crops. Nayar (1975) on treating high yeilding tapioca cultivars with gamma rays and with EMS obtained several mutants and tetraploids. Garlic cloves have been gamma irradiated or treated with chemicals, both treatments resulting in mutations in VM_1 ie. the first cycle of vegetative multiplication after mutagen treatment (Sklyar, 1973). Jacobson (1923) reported considerable increase in yield and larger tubers of two different cultivars of potato using X-rays. Induction of mutations for earliness, increased resistance to different diseases and increased starch content of tubers was reported by Solomko (1962).

It was found possible to use temporary radiation induced partial sexuality in breeding of obligate apomicts, and subsequently select apomictic types with favourable morphological characters (Julen, 1961). Mutants were obtained from the vegetatively propagated, triploid Floratum St.Augustine grass (Stenotaphrum secundatum) (Powell, 1974). In Dallis grass, mutants had been reported by Bashaw and Hoff (1962) and Burton and Jackson (1962).

According to Heinz (1973) the occurrance of chimerism, a common problem on mutation breeding of vegetatively propagated plants, seemed to be limited to two or three vegetative generations, if proper selection was carried out, although Jagatheesan (1976) emphasized that the stabilization of a mutant was the main problem and that more vegetative propagations were needed to assure that the mutant would never revert back to the original type.

Pillai et al. (1974) in a study to isolate superior clones in guinea grass observed a correlation between green fodder yield and leaf stem ratio. Based on the study of 24 diverse varieties of guinea grass Sreenivasan (1983) reported significant positive correlation of characters such as dry weight, leaf area index, plant height, length of panicle, days to fifty percent flowering, girth of internode and crude fibre content on green fodder yield. The association was found negative in the case of crude protein and number of tillers with green fodder yield.

Based on a study in Rhodes grass (Chloris gayana) Boonman (1978) reported that herbage yield was negatively correlated with digestability and leaf stem ratio. In forage sorghum a positive correlation between fodder yield and plant height was observed by Ross et al (1979). Vaithialingam (1979) reported that plant height, leaf area and dry fodder yield were positively correlated with green fodder yield in sorghum.

Isolation of superior clones in species of fodder grasses has been attempted by a few workers. Guptha and Athwal (1966) and Gupta (1968) concluded that high tillering and more leafiness were important for increasing green fodder yield and that stem thickness and plant height were not of much importance, after their extensive studies in pearl millet. Nayar (1979) has reported success in isolating many superior clones of lemon grass in M_1V_1 progeny obtained from M_1 chimeric plants through gamma irradiation. Out of a total 49 clones evaluated eleven gave higher grass yield over the control.

MATERIALS AND METHODS

Plate I. Guinea grass (*Panicum maximum* J.) var. Makuenii

MATERIALS AND METHODS

A. Materials

a. Biological Material

Biological material involved in the present study consisted of var. Makuenii (Plate I) belonging to the most popular fodder grass of Kerala, namely, guinea grass (Panicum maximum J). Makuenii is hirsute with wider leaves than common guinea grass and the canopy is less erect than in other varieties. Annual total dry matter yields of Makuenii are similar to those of other commercial varieties. Although it makes less summer growth, it substantially out yields other varieties in cool season and it is persistant (Annon, 1974).

b. Mutagens

Both physical and chemical mutagens were used for induction of mutation.

i) Physical mutagen

Gamma irradiation was done using ^{60}Co gamma rays from the gamma cell installed at the Radio Tracer Laboratory of the Kerala Agricultural University, Vellanikara. The source was operating at an intensity of 60 Krad/hr. A short range of doses from 15 Krad to 30 Krad at increments of 5 Krad were tried to get maximum incidence of viable mutations.

ii) Chemical mutagens

The most effective chemical mutagen, viz., ethyl methane sulphonate (CH_3SO_2-O-C_2H_3) at four concentration, viz. 0.25%, 0.50%, 0.75% and 1.00% was employed for induction of mutations.

B. Methods

i) Collection and selection of seeds

Seeds of well grown adult plants were collected from the germplasm maintained in the crop museum of the Department of Agronomy Agricultural College, Vellayani. The chosen clumps were allowed to flower profusely and the inflorescence were carefully covered with butter paper covers to prevent shattering of grains and were then collected.

ii) Storage of seeds

Seeds were stored in brown paper covers for allowing them to pass the dormancy phase. Periodical testing of seeds were done to ascertain the maximum viability of seeds. This was done by a separate germination test. Pots of 30 cm width filled with potting mixture of sand, soil and cowdung in 1:1:1 ratio were prepared. Hundred seeds in each storage category, viz. fresh seeds on harvest and 20 days, 40 days, 60 days, 80 days, 100 days, 120 days, 160 days, 180 days, 200 days, 12 months and 18 month old seeds were sown for this study.

iii) Treatment of seeds with gamma rays

Well filled, sturdy, bold seeds which had completed their dormancy phase were hand picked and five gram each of seeds were packed in polythene covers of size 10 cm x 10 cm. The selected seeds were than evenly spread in the covers for getting uniform exposure to radiation. Seeds were irradiated through a single exposure and the doses were regulated by adjustment of time.

iv) Treatment of seeds with EMS

EMS solutions of 0.25%, 0.50%, 0.75% and 1.00% were prepared in glass distilled water. Five gram seeds were used for one concentration. Seeds were presoaked by immersing in double distilled water for four hours. The seeds were then drained and kept moist for another eight hours. The soaked seeds were dropped into conical flasks, containing solutions of EMS at different concentrations. Intermittent shaking was given to facilitate uniform absorption of mutagen by the seeds. The chemical was then drained off after eight hours and the treated seeds were washed in running water several times. The whole treatment was carried out at room temperature.

v) Raising of seeds in nursery

1500 seeds from each treatment of gamma irradiation at the rate of 500 seeds/tray were sown in nursery trays in three replications. On the eighth day of treatment 1500 EMS treated seeds in each treatment were sown immediately after post treatment in trays at the

rate of 500 seeds/trays in three replications. Each replication consisted of a control with untreated seeds. Thus there were nine treatments including control and 3 replications. The treatments were as follows:-

Treatment 1 - Control,
Treatment 2 - 15 Krad gamma rays
Treatment 3 - 20 Krad gamma rays
Treatment 4 - 25 Krad gamma rays
Treatment 5 - 30 Krad gamma rays
Treatment 6 - 0.25 per cent EMS
Treatment 7 - 0.5 per cent EMS
Treatment 8 - 0.75 per cent EMS
Treatment 9 - 1.00 per cent EMS

The EMS treatment was done on the 8th days of gamma irradiation such that the date of sowing of treatments would synchronise and the control was also sown the same day. The seeds were sown in cement trays of 1m x 1m x 30 cm. The pot mixture was made using cattle manure, soil and river sand in ratio 1:2:1. A fine layer of humus rich forest soil was sprinkled on top of trays. After sowing the seeds, a thin layer of sand was spread on top and the trays were regularly watered using watering can.

vi) Raising M_1 generation in main field

The land was thoroughly ploughed and clods removed. Cowdung was incorporated as basal dressing. Drainage channels were provided.

50 day old seedlings were carefully uprooted and transplated to main field. The field experiment was laid out on Randomised Block Design with 9 treatments and 3 replications.

The following observations were recorded in the M_1 generation:

1. Germination of seeds
2. Survival of seedlings
3. Height of seedlings
4. Survival of plants
5. Height of plants
6. Tiller counts
7. Number of inflorescence per hill
8. Pollen sterility
9. Chlorophyll chimeras
10. Morphological abnormalities

1. Germination of seeds

Germination counts of the different treatments were taken from date of sowing in trays. The counts were taken everyday from 5th day upto 10 days and later on at 10 days interval upto 30th day after sowing. The germination counts were taken from 500 seeds sown in one of the trays of every treatment.

2. Survival of Seedlings

Each week the total number of seedlings survived in each treatment was recorded upto 5th week. This observation was recorded from the trays in which germination counts were taken.

3. Height of Seedlings

Ten seedlings were selected at random from each tray of each treatment for recording plant height. Height was measured in centimetres from soil level to tip of shoot, weekly from 2nd week to 5th week. Mean height of each treatment was calculated.

4. Survival of Plants

After transplanting of seedlings to the main field counts on survival were taken twice at 30th and 45th day after transplanting. The mean value was taken and percentage survival of plants over initial transplanted population was calculated.

vii) Raising of M_1V_1 clonal progeny

The M_1V_1 progeny was raised vegetatively from the selected M_1 clumps of the field trial. 50 plants were randomly selected from each M_1 treatment. Slips were separated and were grown as progeny rows under partial shade condition in coconut gardens. In each treatment there were 50 progeny rows, each row representing one plant in the M_1 generation. At 50 per cent flowering stage observations were taken from 20 progeny rows selected at random. From each progeny row 10 plants were selected and studied for the following characters and their mean found out. As enough plants were not available in 30 krad gamma ray treatment and 1.00 per cent EMS treatment, both had to be delected from the study of M_1V_1.

Observations in M_1V_1 generation

1. Number of Tillers
2. Plant Height
3. Girth of internode
4. Days to 50 per cent flowering
5. Leaf area index
6. Yield of green fodder

1. No. of Tillers

The total number of tillers in each hill were counted and recorded. Ten plants from each progeny row were selected for study.

2. Plant Height

The plant height of 10 plants in each progeny row was observed. Height was measured from ground level to top of the shoot and recorded in centimeters.

3. Girth of internode

Girth was taken using vernier calipers. The maximum and minimum diameters of the culm were noted at 5 cm height from base. The girth was finally calculated using formula, $g = 2\pi \frac{\sqrt{a^2+b^2}}{2}$ where 'g' is the girth of the culm, 'a' maximum diameter and 'b' minimum diameter.

4. Leaf area index

Leaf area index is the radio of leaf area (one side) to ground area (Leopold and Kriedemann, 1975). The main tiller was selected for the study. Length and breadth of all leaves of main tiller were measured in centimeters. Area of each leaf was computed using formula A = k x l x w where A = area, l = lamina length, w = maximum lamina width and K = leaf area constant which is computed from actually measured leaf area (Gomez, 1972 in rice, Choudhan et al., 1978 in sorghum and Ferraris and Wood, 1980 in Pennisitum). Here area was found out using graph paper after measuring length and breadth of 10 sample leaves. Leaf area constant for experimental crop was computed by substituting those on formula. Average value for the leaf area constant for a single leaf was obtained to be 0.76 approximately. Average leaf area of a tiller was multiplied with total number of tillers in five sample hills to get total leaf area of five hills. Leaf area index was computed by dividing this value, by land area occupied by five hills.

5. Days to 50 per cent flowering

Number of days taken from the date of planting to the date of emergence of anthers in 50 per cent of plants in each plot was recorded.

6. Yield of green fodder

The mean green fodder yield was calculated from the total green fodder obtained in two cuttings from 10 plants in each progeny row.

RESULTS

RESULTS

The present experiment was conducted with the objective of inducing variability in the guinea grass species Panicum maximum. Jacq. var Makuenii. There were nine treatments, viz.,

Treatment 1	- Control
Treatment 2	- 15 krad gamma rays
Treatment 3	- 20 krad gamma rays
Treatment 4	-25 krad gamma rays
Treatment 5	- 30 krad gamma rays
Treatment 6	- 0.25% EMS
Treatment 7	- 0.50% EMS
Treatment 8	- 0.75% EMS
Treatment 9	- 1.00% EMS

The effects of above treatments were observed in M_1 and M_1V_1 generations and later statistically analysed. The results are given below.

1. Study on germination

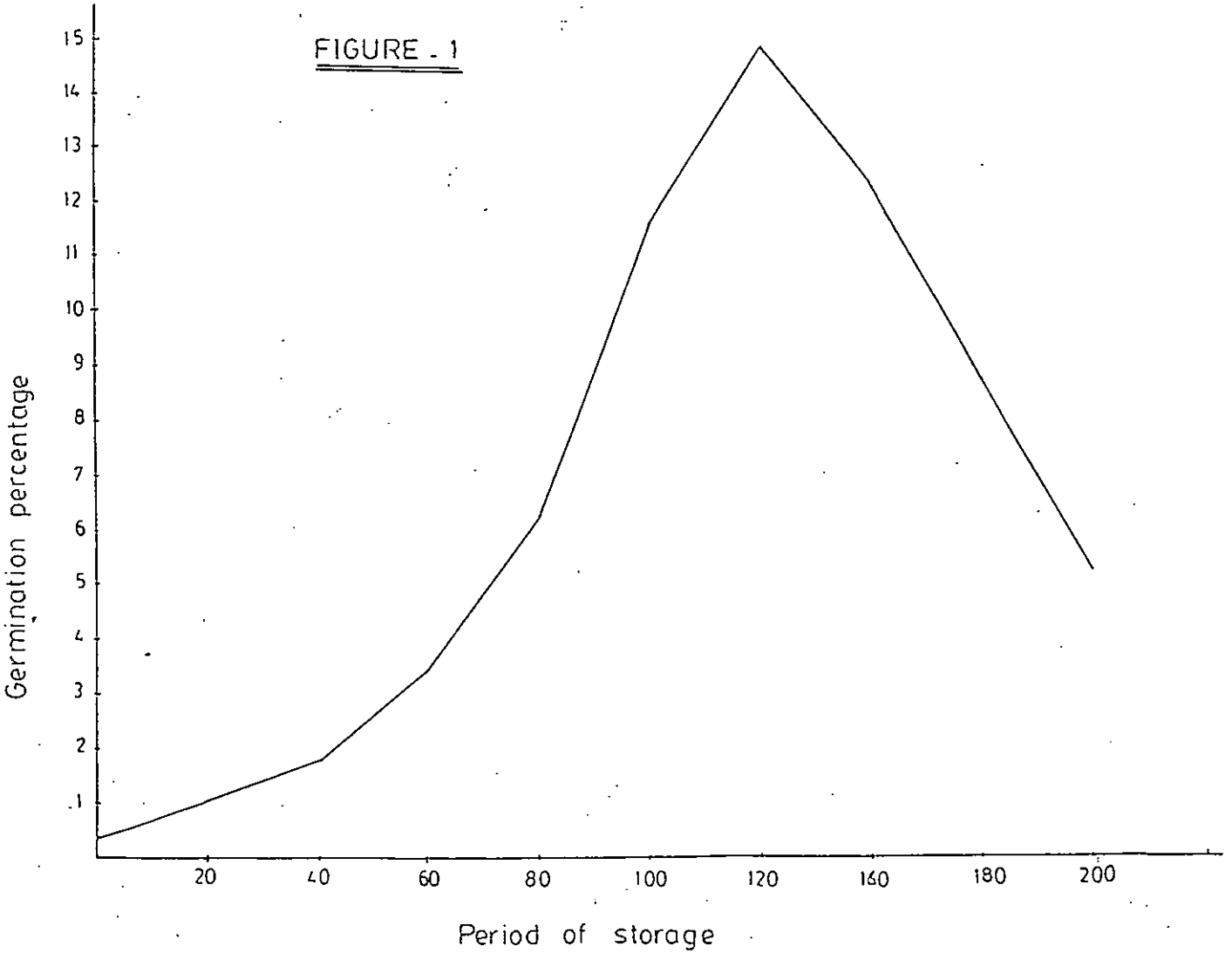
A preliminary study on the influence of storage time on percentage germination of seeds, was conducted following the procedure adopted by Prasad and Gopimony (1982) and the results are given as Table 1 and are graphically presented in Fig.1. Freshly harvested seeds when allowed to sprout were found to have a very low percentage germination value of 0.3%. As the periods of storage increased

Table 1 Effect of storage of seeds on germination

Sl. No.	Period of storage	Germination percentage
1.	Fresh seeds on harvest	0.30
2.	20 days	1.00
3.	40 days	1.80
4.	60 days	3.40
5.	80 days	6.20
6.	100 days	11.55
7.	120 days	14.85
8.	160 days	12.34
9.	180 days	8.64
10.	200 days	5.05
11.	12 months	3.20
12.	18 months	0.80

FIG. 1 Effect of storage of seeds on germination

FIGURE - 1


Germination percentage
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
20
40
60
80
100
120
160
180
200
Period of storage

the viability also increased gradually. The seeds stored for a period of 120 days after harvest recorded a maximum germination of 14.85 per cent. With further increase in storage period germination percentage showed a downward trend. By 18 months the viability decreased to a considerable extent and almost attained viability percentage of freshly harvested ones.

2. Effect of mutagens on M_1 generation

i) Germination of seeds (Nursery)

For the treatments of mutagens, seeds that were stored for 120 days after harvest were used since such seeds were found to have maximum germination. The germination of seeds in nursery trays and their mean values are presented in Table 2. Fig.2 presents the number of seeds germinated in different treatments. The effects of EMS and gamma rays in different periods of time were studied. The Analysis of variances are presented in Appendix I.

Both gamma radiation and EMS treatments were found to have significant effect on seed germination. The lowest dose of gamma radiation, viz., 15 krad was found to have a slight stimulatory effect on germination upto 8th day. However, generally a decrease in germination compared to control was observed in the gamma [illegible] treated seeds. The reduction in germination was found to increase with doses and was most pronounced in 30 krad treatment. In almost all treatments maximum germination was recorded on 10th day and thereafter there was a decline in germination. Observations on 20th and 30th day did not give any significant differences.

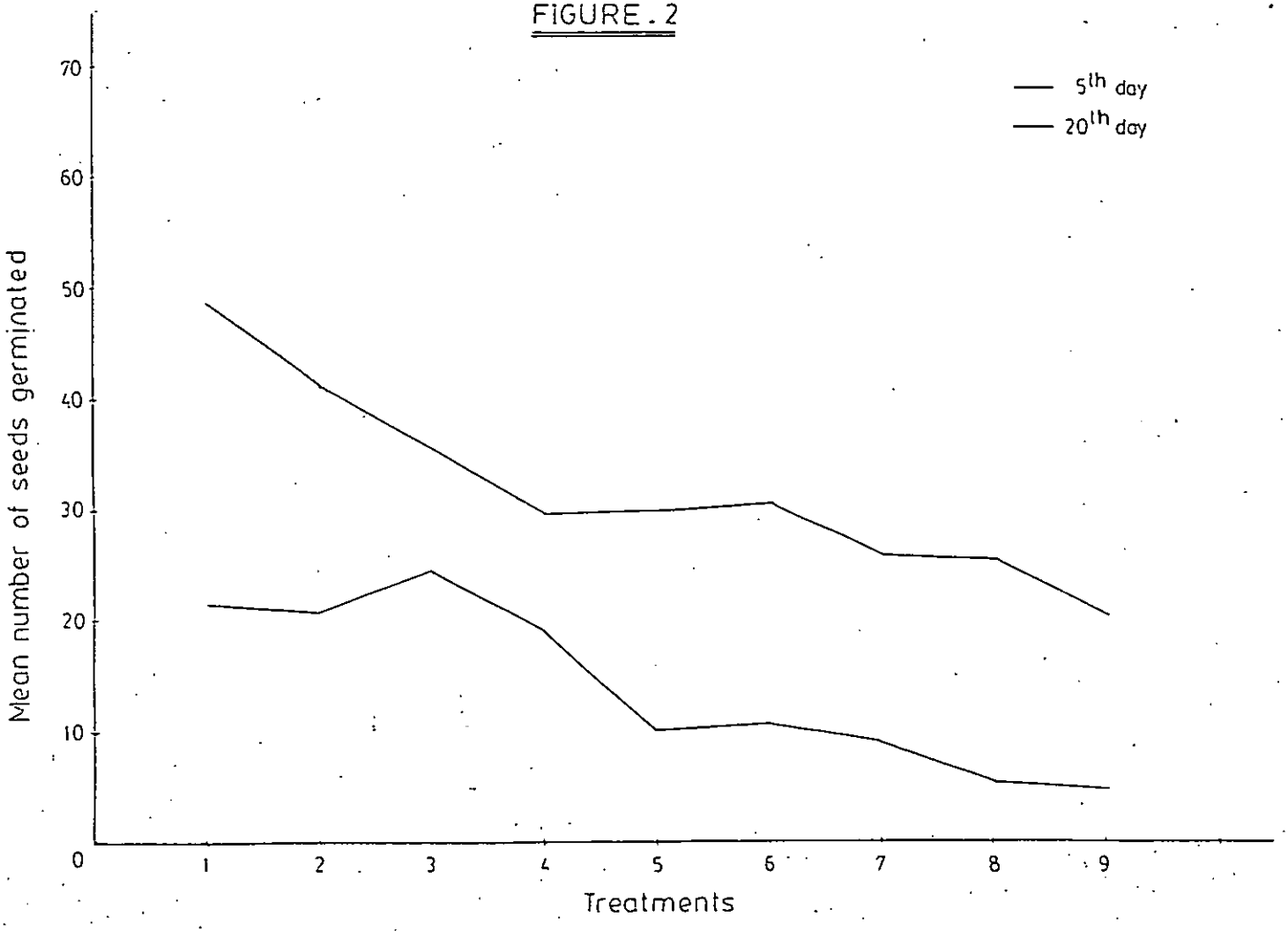
TABLE 2. EFFECT OF MUTAGENS ON SEED GERMINATION IN THE M_1 GENERATION (NURSERY)

Treatment	DAYS								PERCENTAGE OF CONTROL							
	5th	6th	7th	8th	9th	10th	20th	30th	5th	6th	7th	8th	9th	10th	20th	30th
1.	23.23	29.00	38.34	37.86	56.67	69.84	47.90	43.60	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
2.	22.00	27.68	42.76	43.69	54.60	61.90	42.60	36.52	94.70	95.44	111.52	115.37	99.99	99.99	88.93	83.76
3.	24.60	23.40	36.00	40.90	38.61	39.56	26.76	21.62	105.89	103.22	93.89	108.02	68.13	88.68	55.86	49.59
4.	19.00	21.69	32.69	36.50	39.50	35.86	29.62	24.92	81.70	80.70	85.26	96.40	69.70	51.34	61.83	57.16
5.	11.41	17.62	24.53	28.69	35.59	29.69	29.69	23.58	49.06	60.75	63.98	75.78	62.80	42.78	61.98	54.08
6.	12.56	12.96	18.67	19.51	22.51	27.82	31.69	26.58	54.00	44.68	46.69	51.53	39.72	39.83	66.15	60.96
7.	9.17	10.96	14.71	15.69	17.47	24.59	27.53	22.56	39.43	37.79	38.37	41.44	30.83	35.20	57.47	51.74
8.	6.71	11.91	55.61	16.76	18.79	21.86	26.72	20.96	28.85	41.06	40.71	44.27	33.17	31.30	55.78	48.07
9.	5.59	8.59	11.69	14.14	17.15	22.60	21.71	18.83	24.04	29.62	30.49	37.35	30.26	32.36	45.32	43.19
	*Sig	Sig	Sig	Sig	Sig	Sig	N.Sig	N.Sig								
	CD	CD	CD	CD	CD	CD										
	6.83	7.91	11.30	15.52	11.83	12.52										

* At 1% level

FIG 2. Effect of mutagens on seed germination in the M_1 genertion (Nursery)

FIGURE . 2


5th day
20th day
Mean number of seeds germinated
0
10
20
30
40
50
60
70
1
2
3
4
5
6
7
8
9
Treatments

Compared to gamma radiation treatments, EMS treatments had greater inhibitory effect on seed germination. A slight delay in germination was noticed in EMS treatment. The maximum germination was observed around 20th day after which it was found to decline.

3. Effect of mutagen on survival of seedlings

Both the radiation and EMS treatments had deleterious effects on survival of seedlings (Table 3). Mean survival of seedlings in different treatments is graphically presented in Fig.3. Eventhough the percentage of survival of seedlings was higher during early periods in gamma treatments, mortality was at higher rate during subsequent periods. Chemical treatment on the other hand showed a very low germination but a good proportion of the germinated plants survived.

4. Height of seedlings

The effect of mutagens on seedling height in M_1 generation is given in Table 4 and is graphically presented in Fig.4 and the analysis of variance in Appendix I. Significant difference was observed from 2nd week to 5th week in the case of mean seedling height. EMS treatment appeared to have a more drastic effect on seedling height. The seedlings appeared to recover from the shock of treatment and the growth of seedlings seemed to approach normality gradually.

TABLE 3. Effect of mutegens on survival of seedling in the M_1 generation

Treatment	Mean Values - Weekly					Percent of control values				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
1.	38.34	69.23	47.90	44.73	41.96	100.00	100.00	100.00	100.00	100.00
2.	42.76	56.93	42.60	34.59	32.61	111.52	82.23	88.94	77.33	77.72
3.	36.00	36.59	25.76	22.79	20.56	93.89	52.85	53.78	50.95	48.99
4.	32.69	36.18	29.62	25.59	21.45	85.26	52.26	61.84	57.20	51.12
5.	24.53	34.35	28.51	25.59	21.59	63.98	49.62	59.52	57.20	51.45
6.	18.67	31.60	31.69	27.99	22.50	48.69	45.65	66.16	62.58	53.62
7.	14.71	23.69	27.53	23.69	19.45	38.37	34.22	57.47	52.96	46.35
8.	15.61	23.24	26.72	20.99	18.69	40.71	33.57	55.78	46.93	44.54
9.	11.69	19.69	21.70	19.25	14.69	30.49	28.44	45.30	43.04	35.00
	* Sig	Sig	Sig	Sig	Sig					
	CD	CD	CD	CD	CD					
	10.82	12.331	11.186	12.376	13.756					

* At 5 % level

Table 4. Effect of mutagens on seedling height in the M_1 generation (in cm)

Treatment	2nd week	3rd week	4th week	5th week
1	3.91	7.23	18.71	28.61
2	3.24	6.92	20.67	27.83
3	2.97	6.65	18.56	26.45
4	2.94	8.04	22.56	25.65
5	2.41	8.14	21.51	26.42
6	1.89	2.92	8.10	14.35
7	1.50	2.51	9.61	16.16
8	1.20	3.32	10.96	12.02
9	1.15	3.44	6.44	13.76
	* Sig	Sig	Sig	Sig
	CD=1.456	CD=2.791	CD=4.019	CD=9.930

* At 5% level

FIG 3. Effect of mutagens on survival of seedlings in the M_1 generation

FIGURE : 3

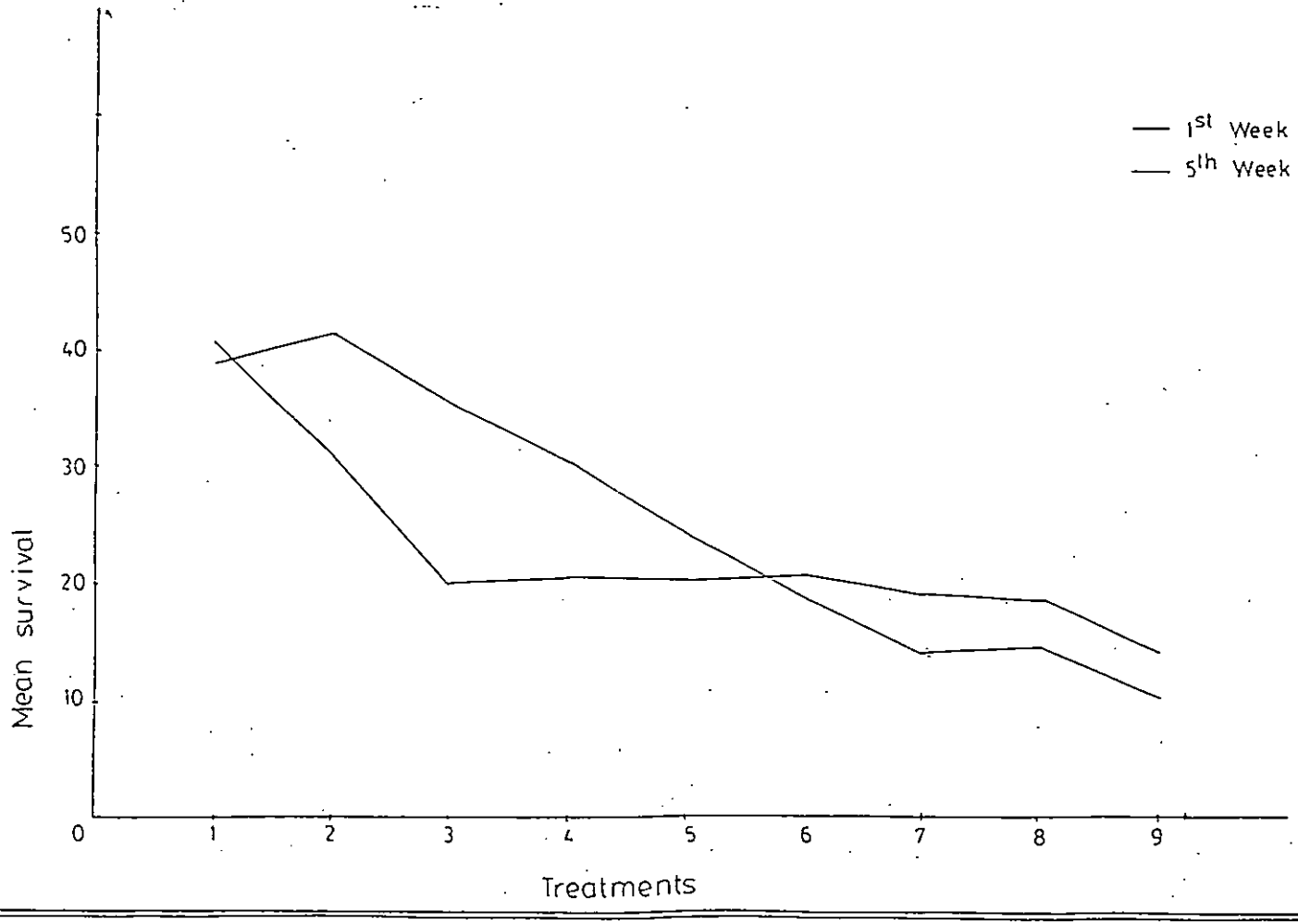
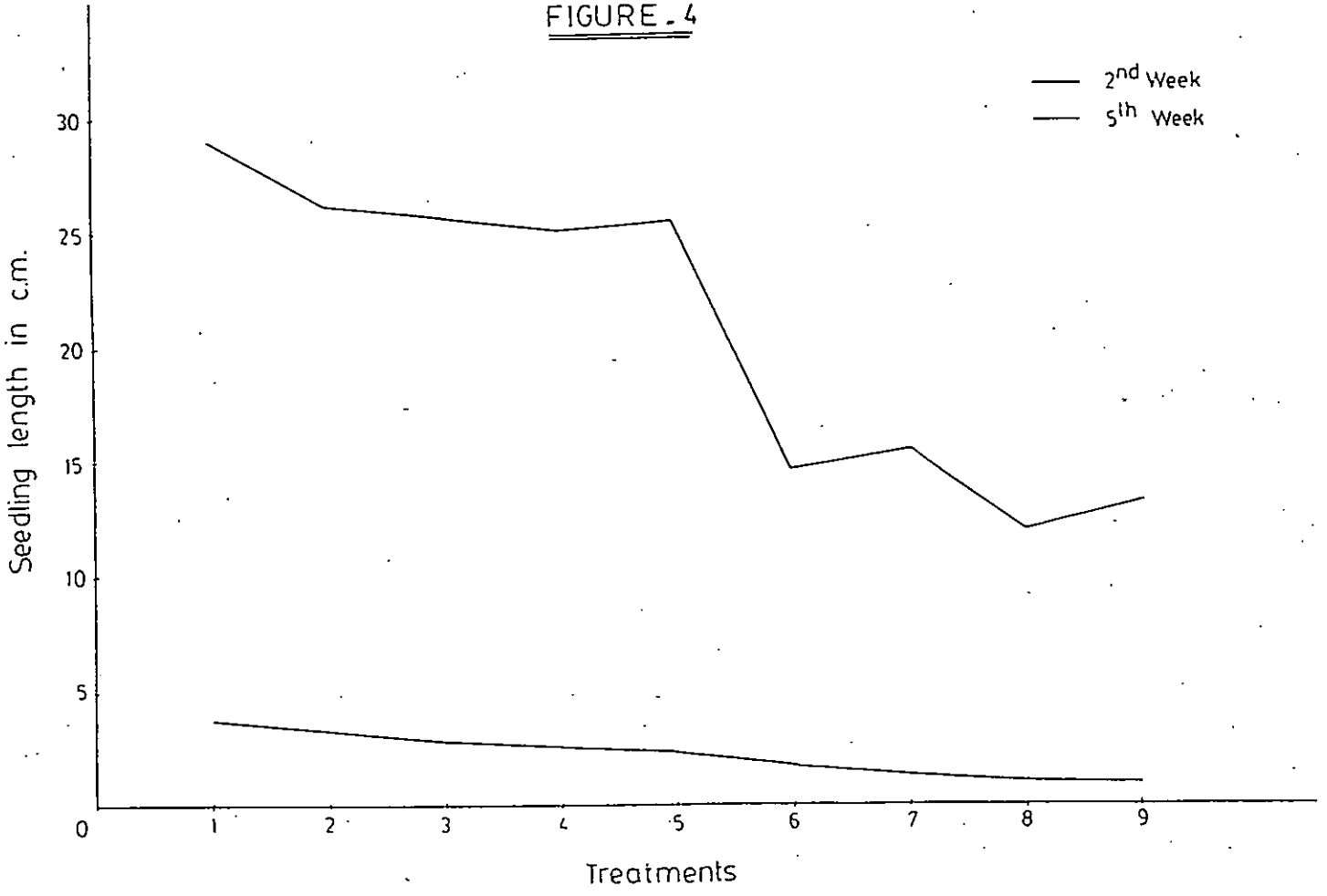

1st Week
5th Week
Mean survival
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Treatments

FIG 4. Effect of mutagens on seedling height in the M_1 generation

FIGURE - 4


2nd Week
5th Week
Seedling length in c.m.
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Treatments

5. Effect of mutagens on survival of plants

Survival of plant is given as percentage survival values over initial transplanted population and the values are tabulated in table 5. Fig.5 represents the percentage survival of plants. The analysis of variance is given as Appendix II. There is significant difference over control in both gamma radiation and EMS treatments during first and second observations. A gradual reduction in survival percentage values was noted as concentration increased in both gamma radiation and EMS during the first month. EMS treatment showed a more drastic reduction in survival during the first month. But these values remained almost unchanged during the second month and the decrease was not much promounced. In the case of gamma radiation a considerable decrease in survival values over first month was seen during the second month.

6. Effect of treatments on height of plants

The effect of treatments on height of plants in main field is given in Table 6 and is presented graphically in Fig.6 and the analysis of variance as Appendix II. Both gamma radiation and EMS produced only a nonsignificant effect on height during first month. Plant height variations showed significant difference when these plants reached 45th days after transplanting. While higher doses of both the physical and chemical mutagens had adverse effect on plant height the 15 krad and 20 krad of gamma irradiation and 0.25% and 0.50% of EMS did not have any effect on the plant height. The maximum

Table 5. Effect of mutagens on survival of plants

Treatment	Percentage survival values over initial transplanted population	
	30th day after transplanting	45th day after transplanting
1	84.62	79.96
2	82.93	81.62
3	74.63	64.94
4	52.98	44.69
5	42.19	30.91
6	44.59	44.61
7	36.59	33.59
8	37.16	32.89
9	24.69	19.59
	* Sig	Sig
	CD=8.49	CD=21.86

* At 1% level

Table 6. Effect of treatments on height of plants

Treatment	Height in cm	
	30 days after transplanting	45 days after transplanting
1	38.17	71.56
2	32.60	69.63
3	34.70	70.62
4	31.60	68.79
5	32.80	54.96
6	38.56	71.89
7	40.79	69.91
8	39.63	62.89
9	37.69	50.49
	N.Sig	* Sig
	CD= 25.97	CD = 39.69
	* At 1% level	

FIG 5. Effect of mutagens on survival of plants

FIGURE - 5

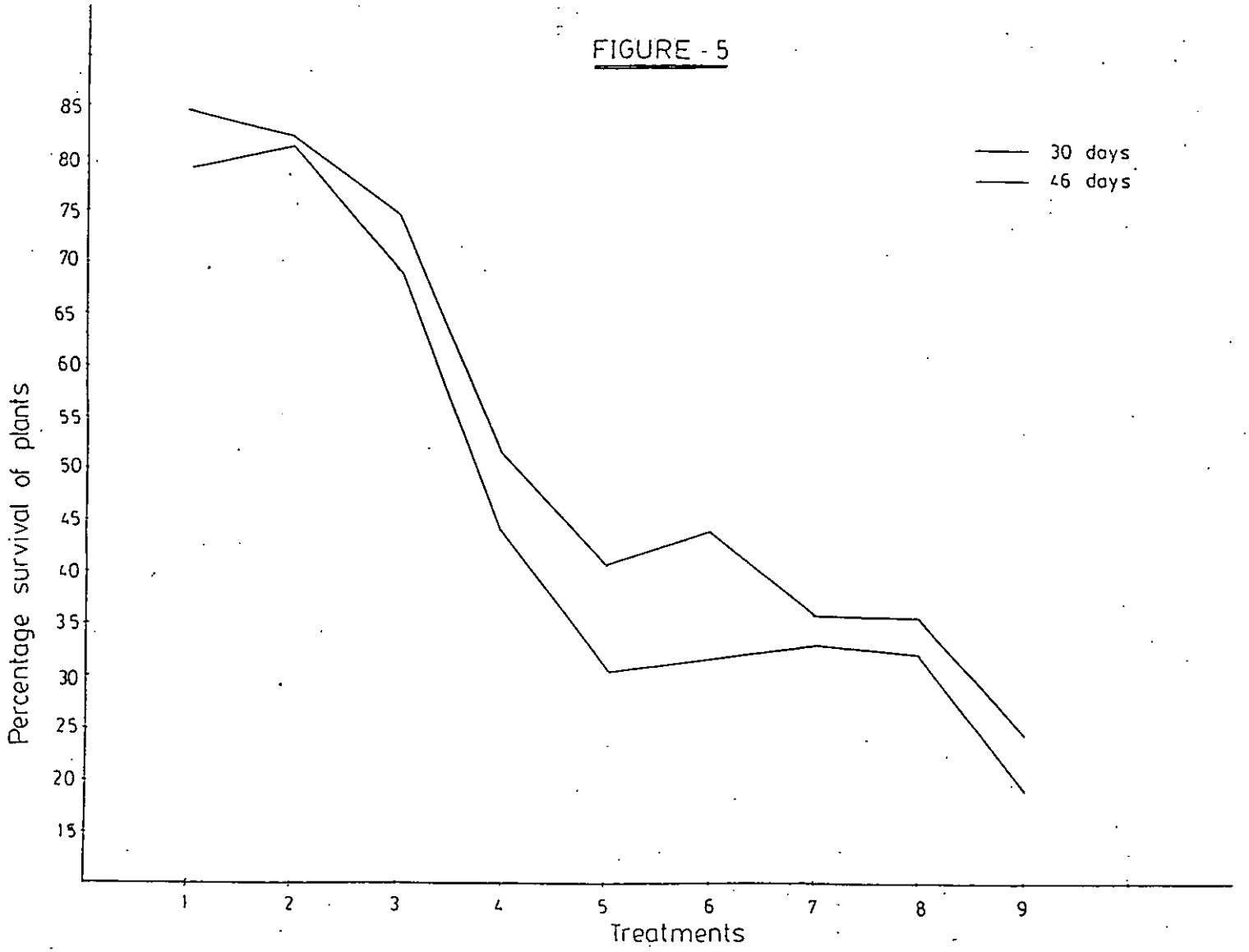
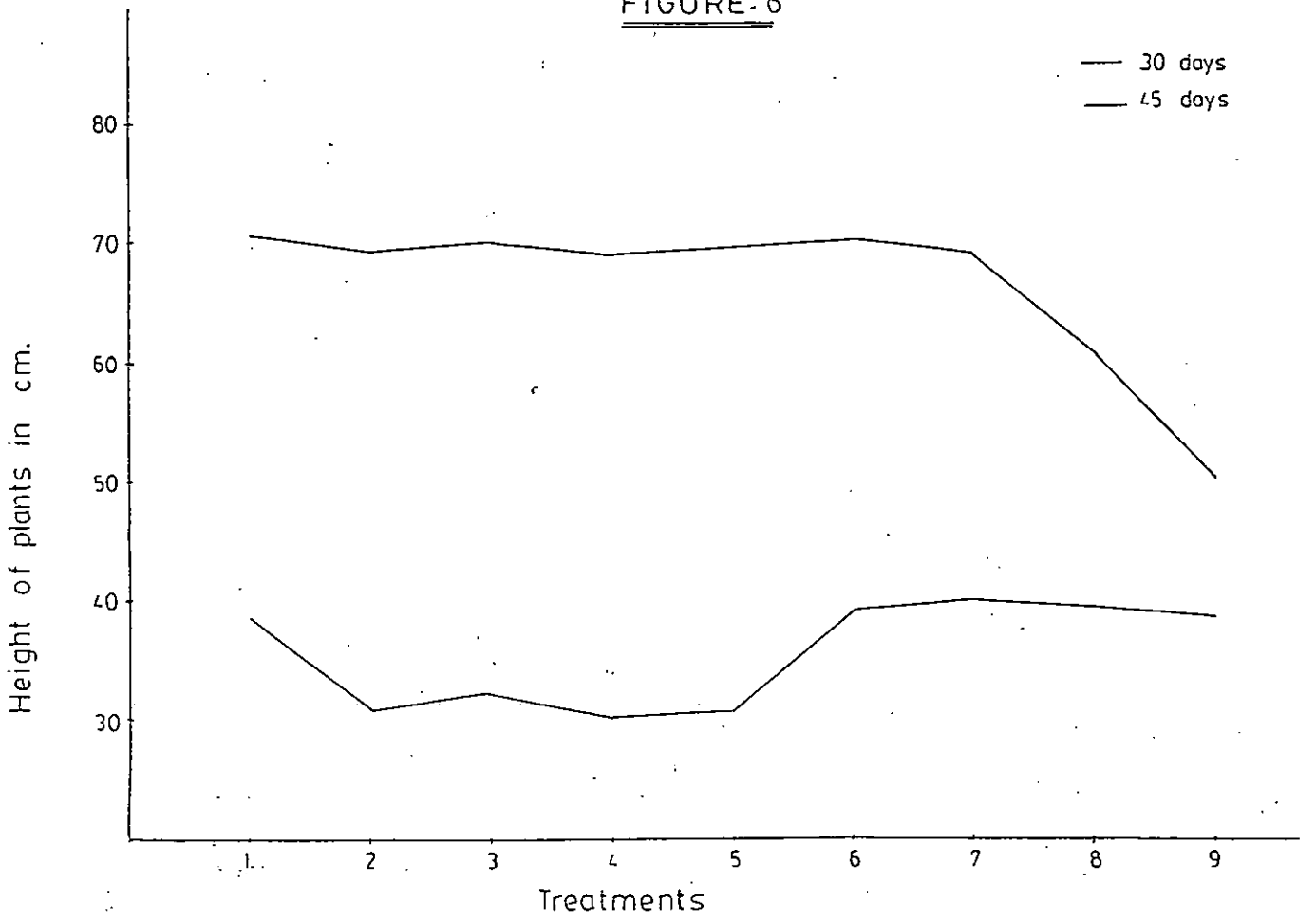

Percentage survival of plants
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
1
2
3
4
5
6
7
8
9
Treatments
30 days
46 days

FIG 6. Effect of treatments on height of plants

FIGURE-6


30 days
45 days
80
70
60
50
40
30
Height of plants in cm.
1
2
3
4
5
6
7
8
9
Treatments

height reduction was obtained in treatment Nos 5 and 9, ie. 30 krad gamma radiation and 1.00% EMS.

7. Effect of treatment on tiller count

The observations are given on Table 7 and analysis of variance on appendix II. The graphical representation of the effect treatments on tiller counts is given in Fig.7. The treatments did not have any effect on the production of tillers in the plants. Observations at 46th day and 55th day showed insignificant difference in tiller counts. However, at both observations the maximum count was recorded by the untreated control plants. The least tiller count was recorded by the treatment 9, viz; EMS at 1.00% concentration. Plate II represents a variant with single tiller in treatment 9. Plates III and IV are the photographs of shy tillering types induced by mutagens.

8. Effect of treatments on number of inflorescence

The observations on number of inflorescence is given in Table 8 and Analysis of variance in Appendix II. Fig. 8 shows the graphical representation of the number of inflorescence in different treatments Plates V and VI depict the effect of mutagens on the inflorescence.

Analysis of data recorded during early stages of growth did not show any significant effect of treatments on the number of

Table 7. Effect of treatments on tiller counts

Treatments	No. of tillor counts	
	1st observation	2nd observation
1	12.35	23.24
2	11.75	23.25
3	12.11	24.32
4	9.99	22.89
5	12.32	18.12
6	8.91	24.23
7	10.81	19.18
8	12.05	17.52
9	11.60	13.21
	N.S.	N.S.
	CD = 6.35	CD = 8.97

FIG 7. Effect of treatments on tiller counts

FIGURE-7

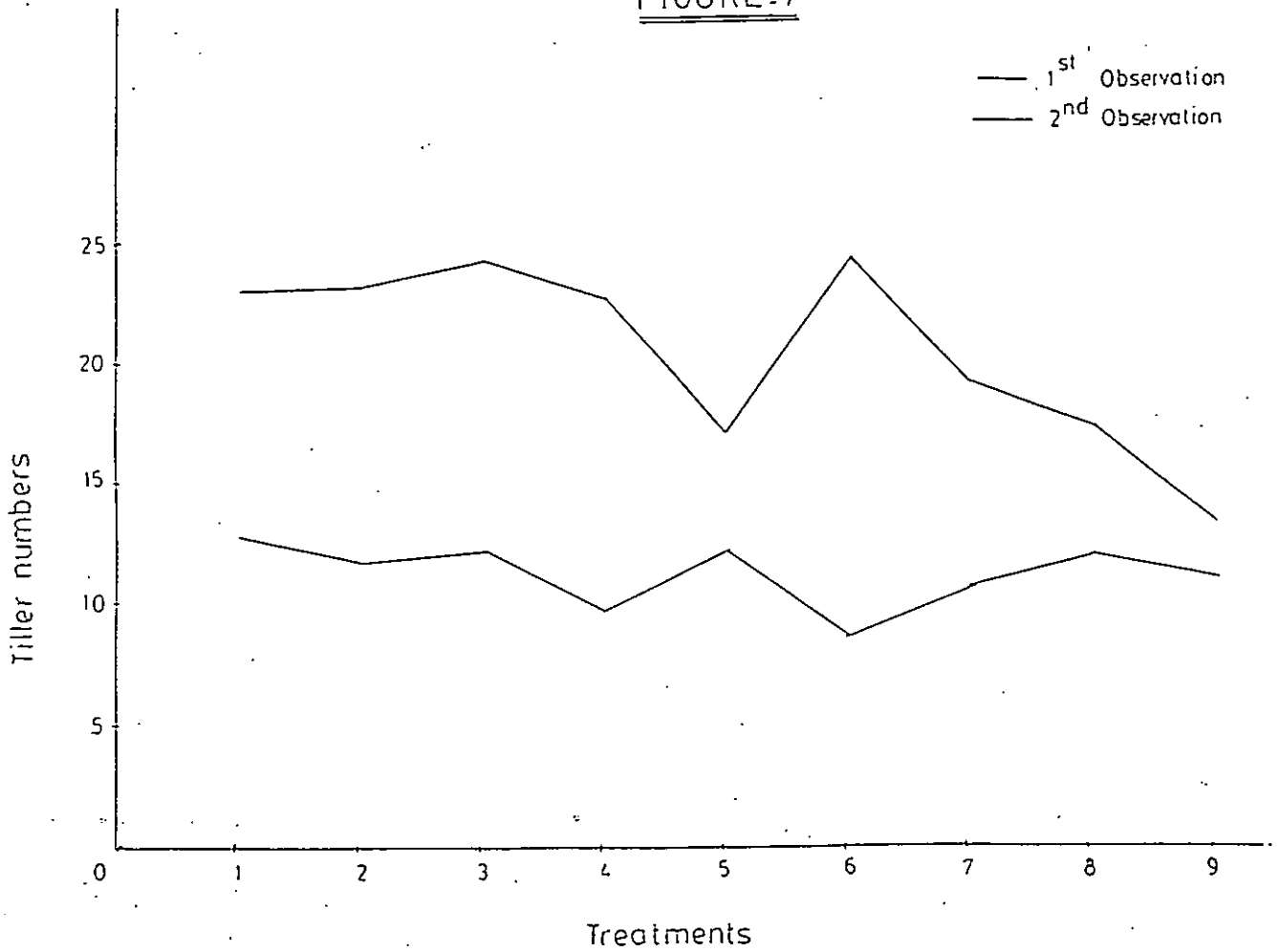

1st Observation
2nd Observation
Tiller numbers
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
Treatments

Table 8. Effect of treatments on inflorescence counts

Treatments	No. of inflorescence/clump	
	1st observation	2nd observation
1	10.17	22.19
2	9.52	21.43
3	8.37	19.79
4	9.61	20.33
5	7.67	15.16
6	10.45	13.47
7	4.80	14.13
8	5.93	10.12
9	7.71	9.68
	N.S.	* Sig
	CD = 6.35	CD - 8.97
	* At 1% level	

FIG 8. Effect of treatments on inflorescence counts

FIGURE - 8

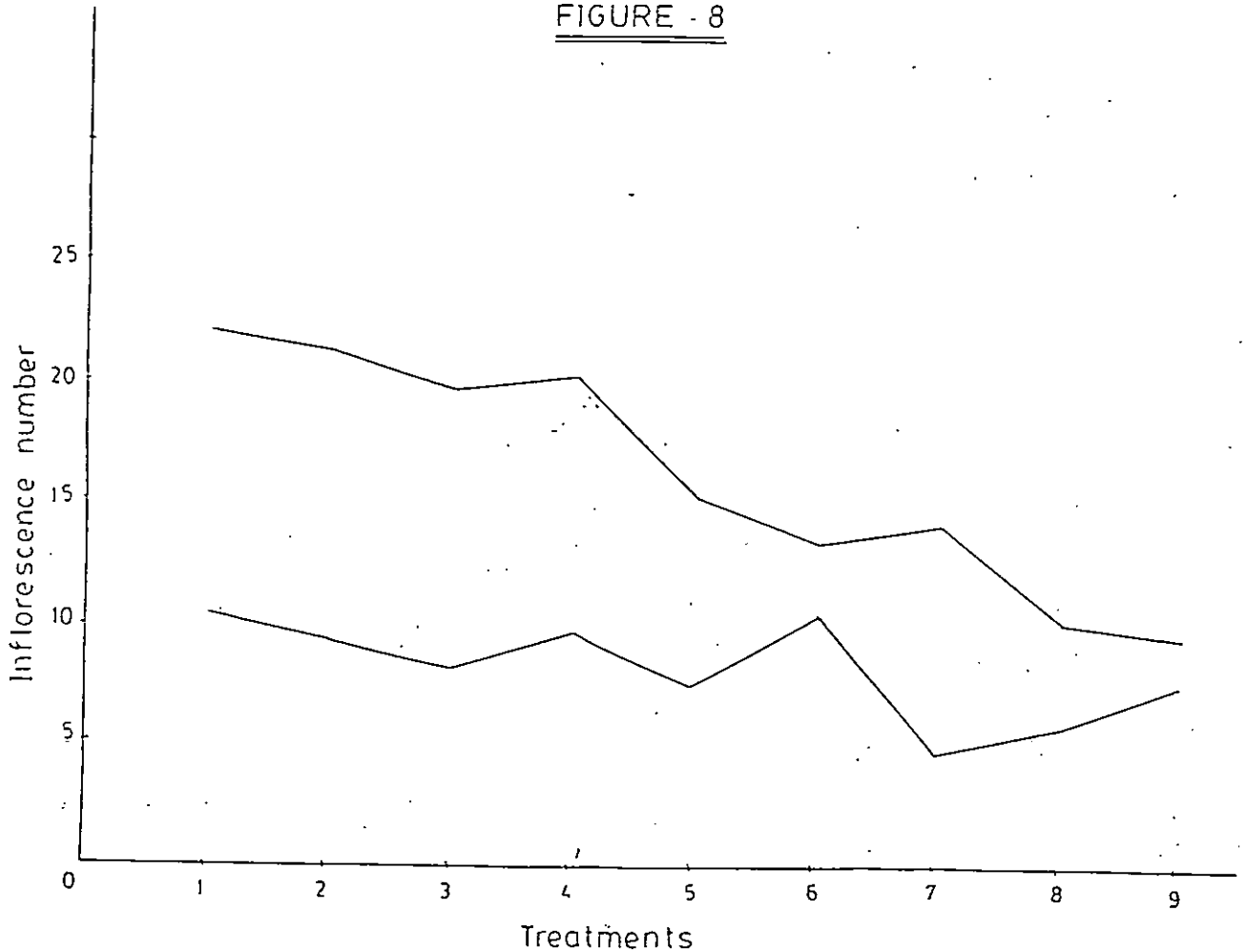


Plate II. Single tiller type

FIGURE - 8

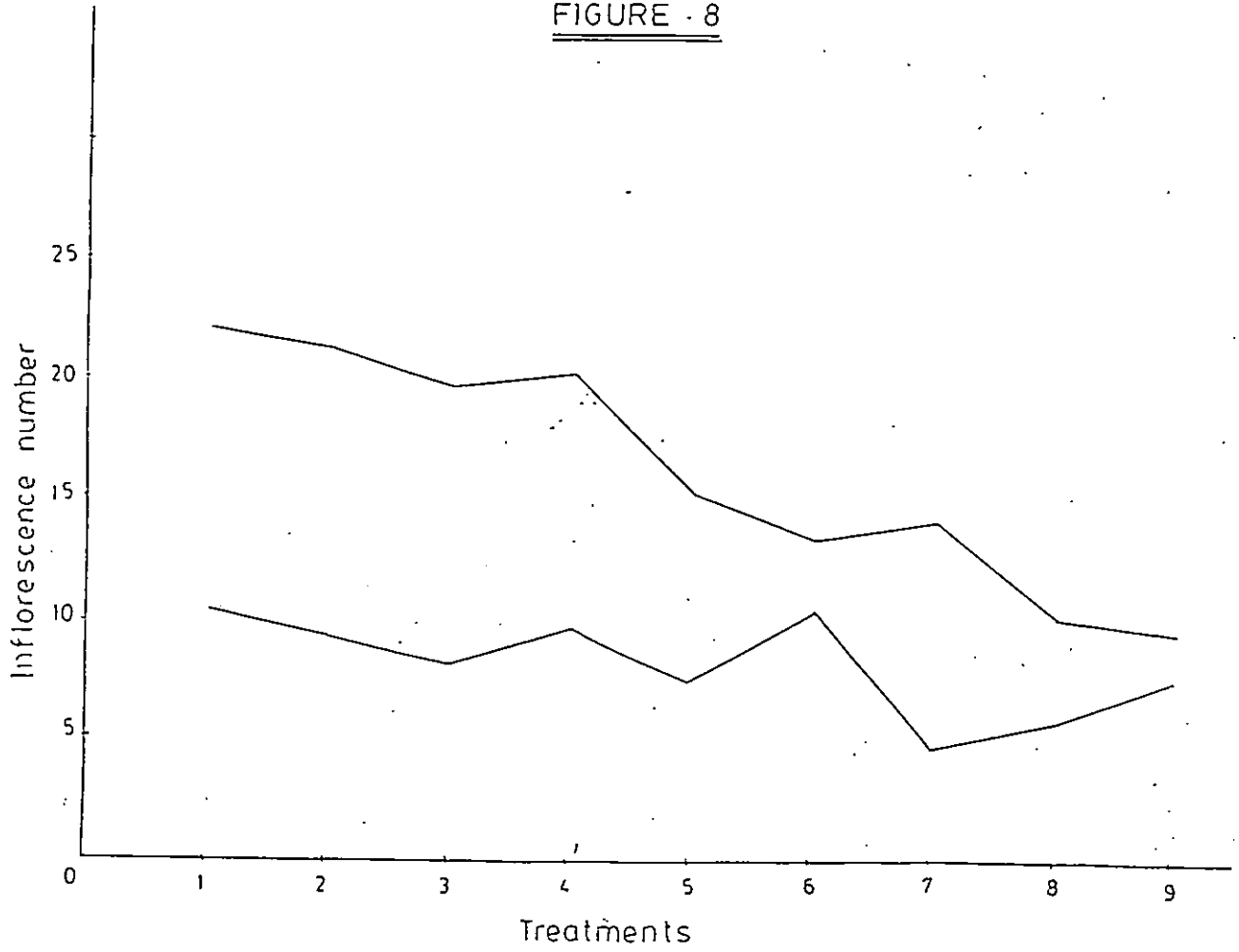


Plate II. Single tiller type

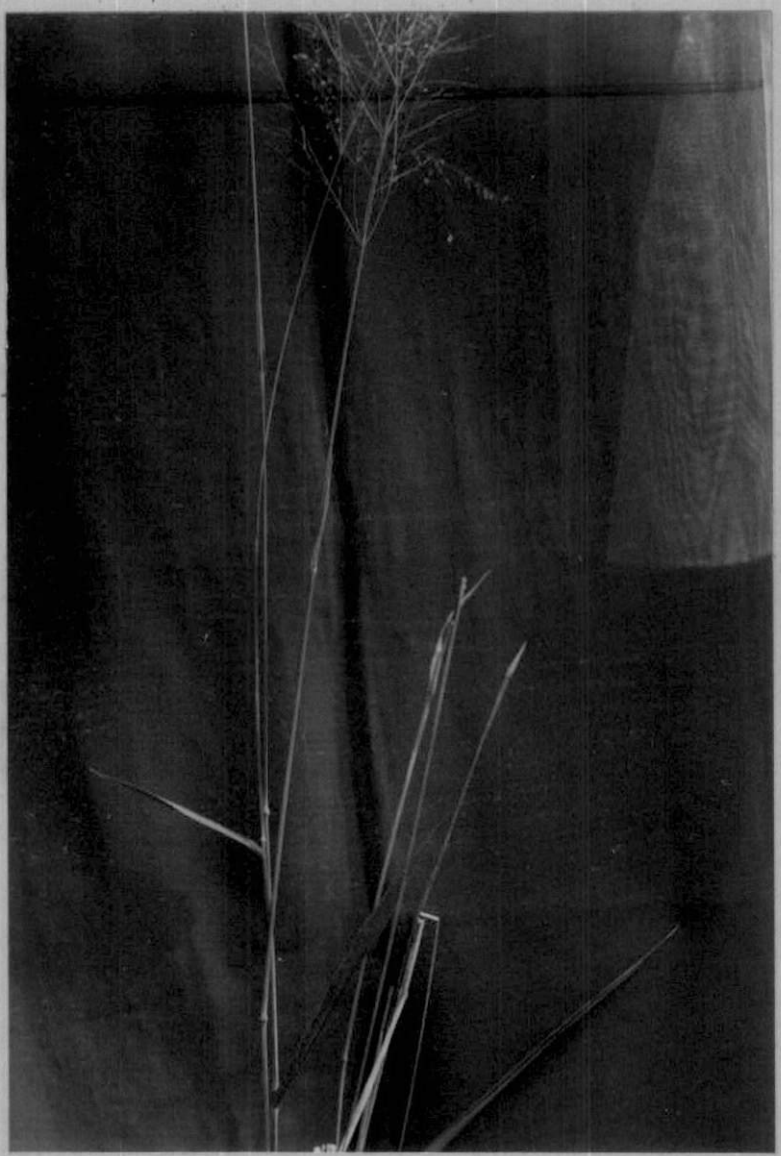


Plate III. Shy tillering types induced by EMS

Plate IV Shy tillering types induced by gamma irradiation

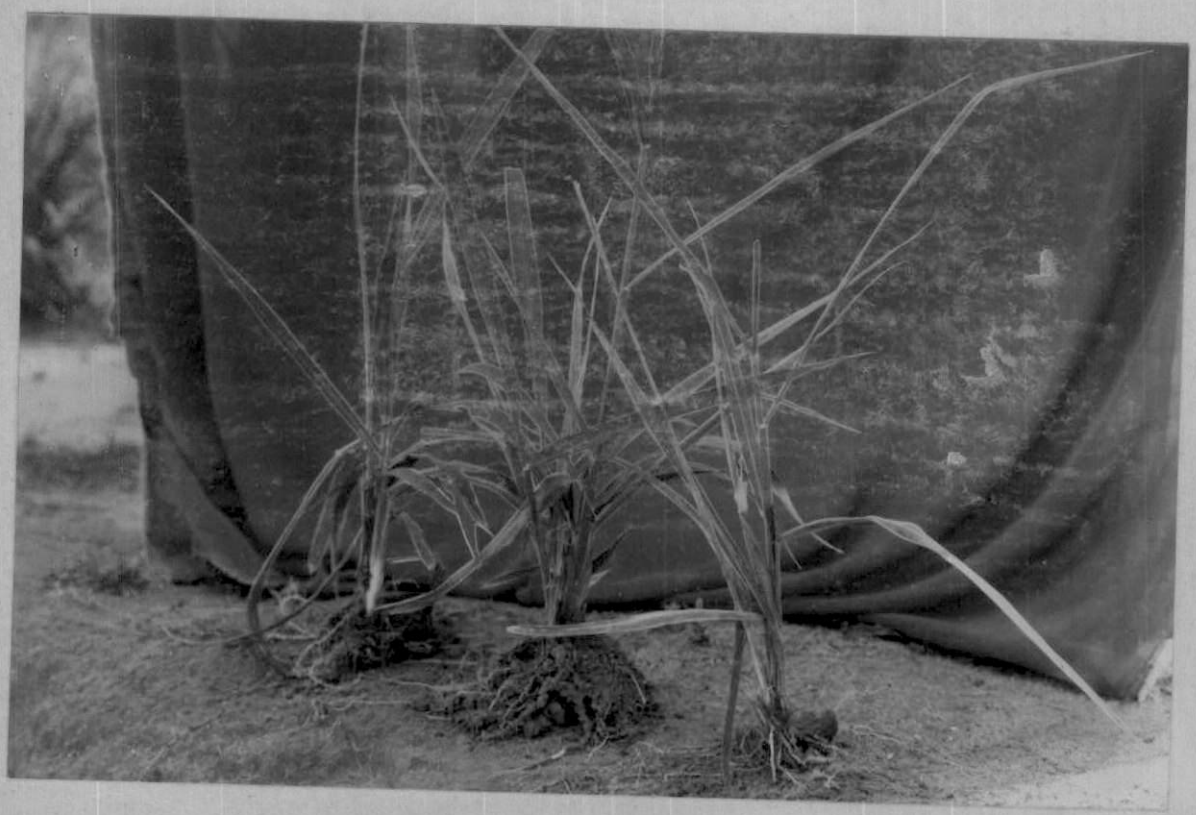


Plate V Gamma ray induced inflorescence types

Plate VI EMS induced inflorescence types

inflorescences. Eventhough the effect is not statistically significant at the first observation, higher doses induced less flowering as indicated by reduced number of inflorescences. The lowest count was recorded by the highest doses of mutagens. During the second observation at later stages of growth the number of inflorescence appeared to show significant difference. Here again, the lowest values were recorded by the highest doses of mutagens. Between gamma radiation and EMS, the chemical treatment showed more inhibitive effect on flowering.

9. Effect of treatments on pollen sterility

The percentage sterility observed is tabulated in Table 9 and is graphically presented in Fig.9. The analysis of variance is given in Appendix II.

Very high pollen sterility was induced by the mutagens. As the dose of mutagens is increased the sterility steadily increased. This is true for both the gamma irradiation and EMS. However gamma rays caused more sterility compared to EMS.

10. Effect of treatments on chlorophyll Chimeras

Hundred M_1 seedlings were studied on each of the treatments to score the occurrence of chimeras and the percentage were worked out. The data are presented in Table 10. Lower doses of the mutagens appeared to produce more number of chlorophyll chimeras.

Table 9, Effect of treatments on pollen sterility

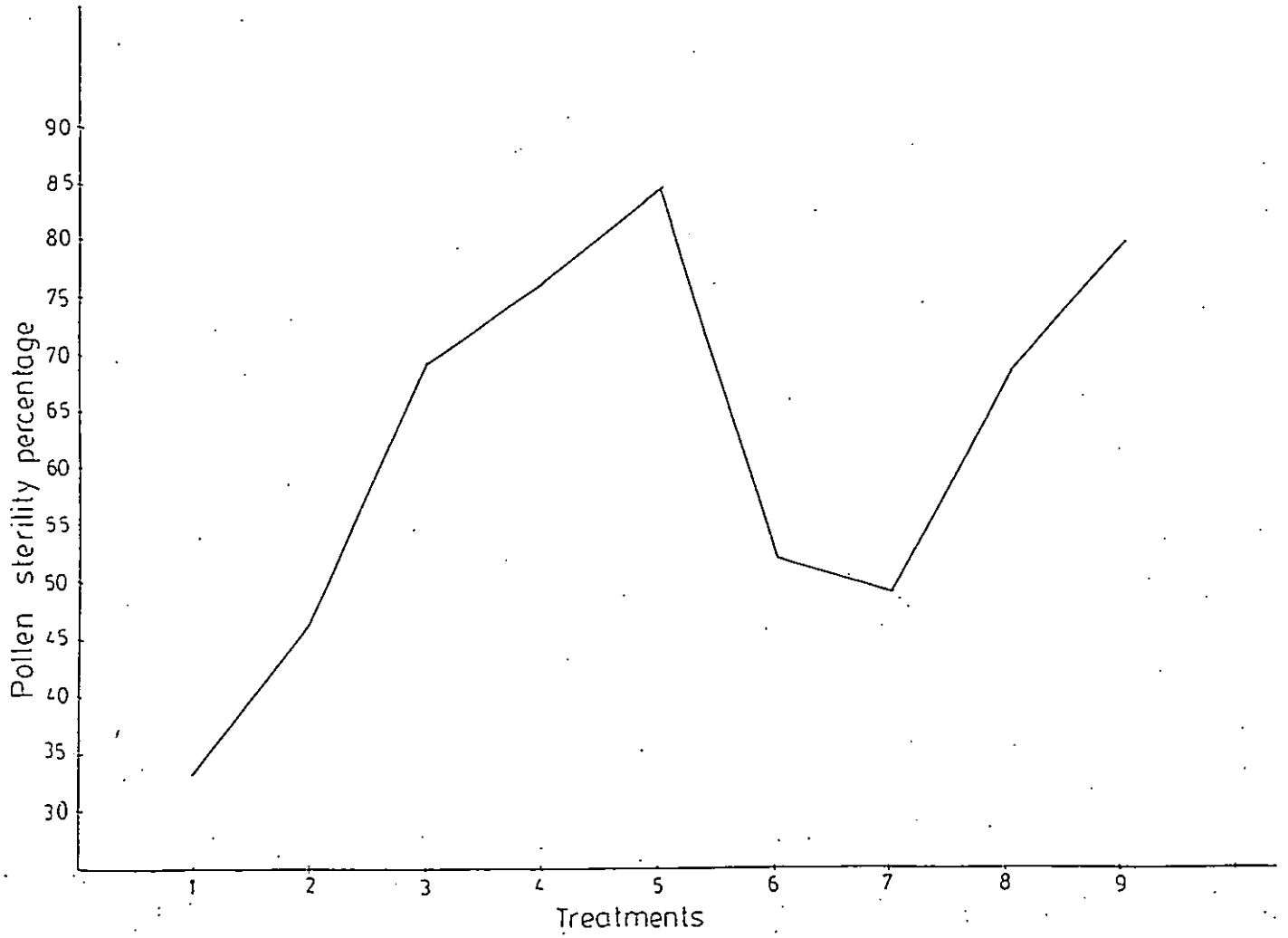
Treatment	Pollen sterility	
	Per cent	Percentage of control
1	33.31	100.00
2	46.55	139.65
3	69.25	207.75
4	76.85	230.55
5	84.62	253.86
6	52.65	157.95
7	49.09	147.27
8	68.45	205.35
9	79.44	238.32
	* Sig	
	CD = 23.451	
	* At 1% level	

Table 10. Effect of treatments on frequency of chlorophyll chimeras in the M_1 generation

Treatment	No. of M_1 seedlings scored	No. of chlorophyll chimeras	Percentage of chimeras
1	100	0	-
2	100	4	4
3	100	2	2
4	100	1	1
5	100	1	1
6	100	5	5
7	100	3	3
8	100	2	2
9	100	2	2

FIG 9. Effect of treatments on pollen sterility

FIGURE . 9


Pollen sterility percentage
90
85
80
75
70
65
60
55
50
45
40
35
30
1
2
3
4
5
6
7
8
9
Treatments

11. Effect of treatments on Morphological abnormalities

The nature of abnormalities produced and their frequency are presented in Table 11. Tall and dwarfs, chaffy earhead, narrow and broad leaves are the various abnormalities noted. Open and semi open types were also observed (Plates VII, VIII, IX and X).

12. Number of Tillers

The data are presented on table No.12. The number of tillers in the M_1V_1 generation did not show significant difference from population mean. The progeny rows within each of the treatments also did not reveal any significant difference among them. The treatment means were almost equal to the population mean on the higher doses of EMS. However, other treatments recorded a reduction in tillering ability, eventhough the difference were not significant.

13. Effect of treatments on height of plants

The data on the height of plants in M_1V_1 generation is recorded in Table 13. The differences in the mean height of plants were significant statistically. The maximum height increase was recorded on 20 krad treatment. While the EMS treatment reduced the plant height, a positive shift on the means was noticed on treatments with gamma rays at 10 krad and 20 krad doses. However, the differences among the progeny rows within each of the treatments were not significant.

Table 11. Effect of treatment on nature of morphological abnormalities caused

Treatment	Nature of Morphological abnormalities observed				
	Tall	Dwarf	Chaffy earhead	Narrow leaved	Bread leaved
2	1		1		1
3	4				
4		1		2	
5				1	
6	4				
7		1		1	2
8		3		2	
9			1		

Table 12. Number of Tillers

Treatment	Number of Tillers
Control	38.10
15 krad	33.97
20 krad	34.50
25 krad	35.50
0.25%	34.85
0.50%	39.73
0.75%	36.82

F value for treatment - 1.44 ns

F value for progeny rows - 1.50 ns

ns - Not significant

Table 13. Height of Plants

Treatments	Height of plants (in cms)
Control	177.44
15 krad	182.82
20 krad	184.25
25 krad	138.25
0.25%	170.57
0.50%	168.01
0.75%	133.77

F value for treatment - 78.532**

F value for progeny rows within Treatments = 0.895 ns

** - Significant at 1% level

ns - Not significant

Plate VII Open type induced by gamma irradiation

Plate VIII Highly open dwarf mutant induced by EMS

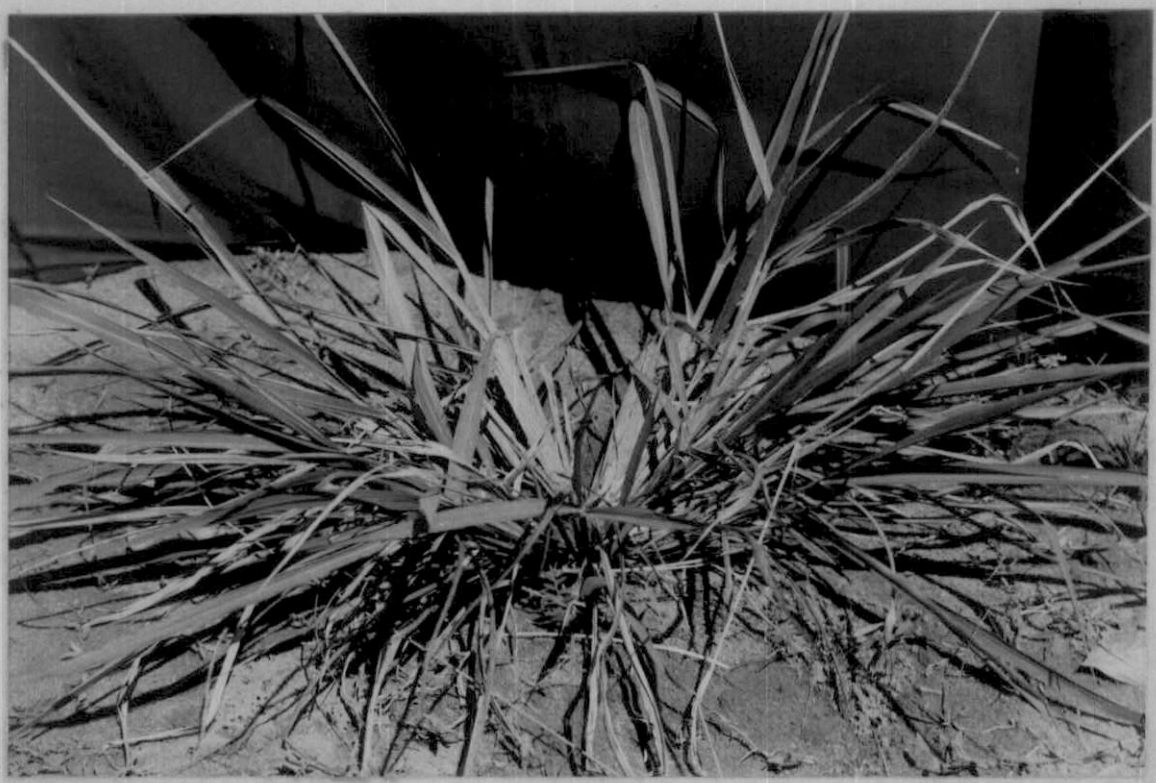
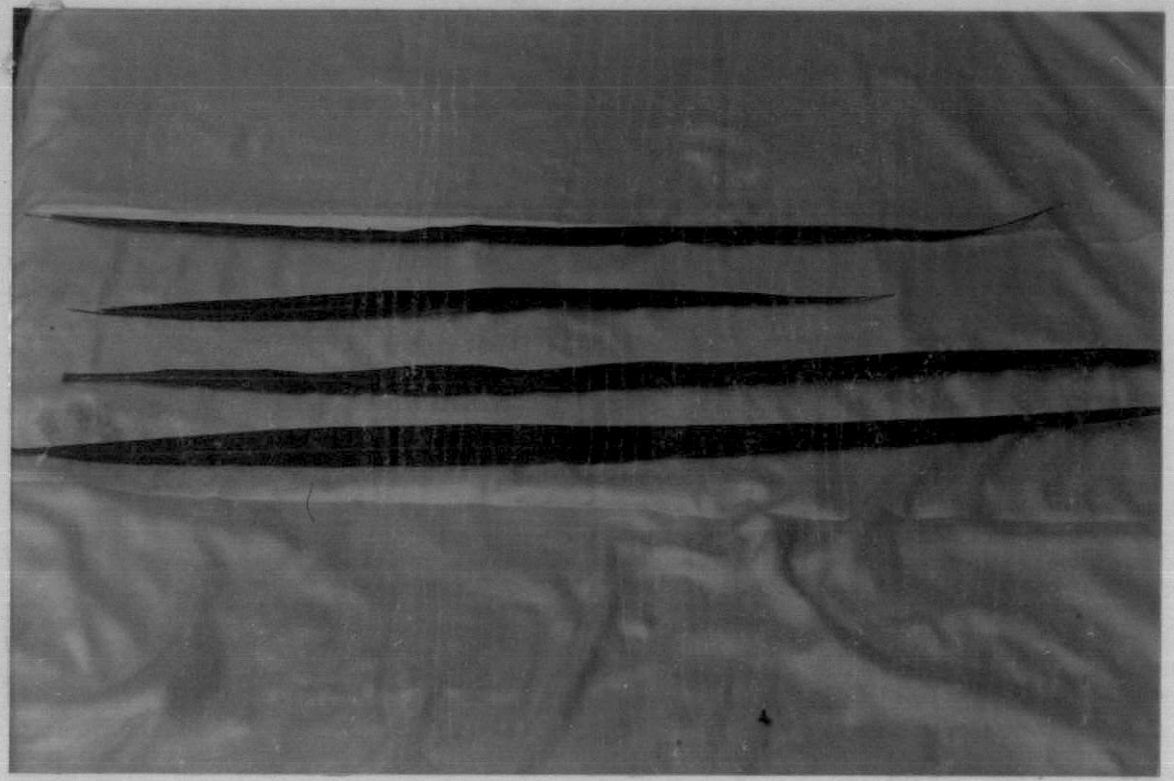


Plate IX Dwarf mutant with single inflorescence

Plate X Leaf Variations induced



14. Effect of treatments on Girth of Internode

Tables 14(a) and 14(b) contain the mean girth of internode treatment wise and progeny-wise respectively. The statistical analysis recorded significant differences among the treatments and among the progences within the treatments. The maximum girth was recorded by 20 krad of gamma irradiation followed by EMS at 0.25% concentration. Among the 20 clones studied in 20 krad treatment while five recorded maximum girth of more than 0.50 cm, only three clones had girth below that of the control clones. All others had either girth thickness equal to or more than that of the control. In 0.25% EMS treatment only one clone recorded girth thickness below that of the control clones.

15. Effect of treatments on days to 50% flowering

The data on days to 50% flowering on M_1V_1 generation is given in Table 15. Flowering time did not appear to have been influenced by the treatment.

16. Effect of treatments on leaf area index

The data on leaf area index are presented in table 16(a) The Table 16(b) contains the progeny-wise results. The leaf area index of treatment plants on M_1V_1 generation is found to be statistically significant over the population mean. The maximum leaf area index recorded in 20 krad gamma irradiation was8.02 cm. The

Table 14(a). Girth of Internode

Treatment	Girth of Internode (cm)
Control	0.350
15 krad	0.326
20 krad	0.443
25 krad	0.296
0.50%	0.335
0.25%	0.420
0.75%	0.304

F value for treatment = 7.237**

F value for progeny rows = 2.188**

** - Significant

TABLE 14 (b). Girth of Internode in cm

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
Control	0.34	0.38	0.35	0.40	0.44	0.40	0.36	0.49	0.28	0.35	0.27	0.32	0.30	0.37	0.43	0.40	0.40	0.21	0.37	0.15	0.350
15 Krad	0.42	0.36	0.32	0.30	0.35	0.36	0.33	0.32	0.25	0.38	0.37	0.37	0.44	0.42	0.26	0.23	0.23	0.33	0.29	0.25	0.326
20 Krad	6.45	0.43	0.46	0.48	0.52	0.45	0.48	0.47	0.51	0.52	0.50	0.46	0.39	0.42	0.36	0.34	0.50	0.33	0.47	0.31	0.443
25 Krad	0.15	0.27	0.23	0.29	0.38	0.31	0.32	0.33	0.34	0.32	0.29	0.30	0.36	0.39	0.36	0.26	0.29	0.23	0.21	0.30	0.296
0.25% EMS	0.37	0.40	0.45	0.51	0.52	0.44	0.48	0.51	0.44	0.34	0.44	0.38	0.25	0.43	0.38	0.36	0.51	0.40	0.35	0.44	0.420
0.50% EMS	0.42	0.43	0.38	0.38	0.29	0.39	0.48	0.34	0.41	0.24	0.36	0.32	0.46	0.42	0.41	0.23	0.22	0.42	0.30	0.23	0.355
0.75% EMS	0.32	0.30	0.31	0.33	0.29	0.29	0.39	0.49	0.26	0.20	0.18	0.28	0.40	0.32	0.21	0.25	0.26	0.38	0.32	0.33	0.304

F value for treatment = 7.237**

F value for progeny rows= 2.188**

** Significant at 1% level.

Table 15. Days to 50% flowering

Treatment	Days to 50% flowering after transplanting
Control	34.185
15 krad	36.421
20 krad	34.699
25 krad	35.151
0.25%	34.529
0.50%	38.074
0.75%	34.039

F value for treatment = 0.590 ns

F value for progeny rows = 0.7511 ns

ns - Not significant

Table 16(a). Leaf Area Index

Treatment	Leaf Area Index
Control	5.93
15 krad	4.75
20 krad	9.02
25 krad	3.62
0.25%	7.81
0.50%	5.53
0.75%	5.49

F value for treatment = 4.574**

** - Significant at 1% level

Leaf Area Index

TABLE 16 (b).

Treatment	Progeny rows																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
Control	6.49	5.86	3.89	5.39	7.35	5.89	4.86	6.45	7.32	5.19	5.86	4.89	7.19	6.36	6.89	5.13	7.98	6.89	4.34	5.89	5.92
15 Krad	3.89	5.62	6.89	4.69	5.89	3.42	2.91	3.86	4.69	5.44	6.29	7.01	2.89	6.45	7.41	4.32	5.63	3.18	2.32	3.19	4.75
20 Krad	7.32	7.63	8.76	7.29	9.05	6.65	8.62	7.89	9.86	9.89	9.32	6.49	5.32	8.61	6.49	8.81	9.89	8.55	5.69	8.76	8.02
25 Krad	3.19	5.86	4.19	2.32	4.89	2.89	3.65	3.41	2.19	4.89	5.32	3.19	2.89	3.45	4.19	3.95	2.82	4.45	4.00	2.31	3.62
0.25% EMS	7.36	6.42	5.19	9.15	9.01	6.89	5.86	9.31	5.32	7.71	8.62	5.89	6.81	7.62	7.98	6.19	9.32	8.98	6.89	8.65	7.81
0.50% EMS	4.18	6.72	5.39	4.89	6.49	7.32	4.31	6.69	5.86	4.32	6.09	4.89	5.36	3.45	6.19	5.45	3.89	6.69	3.19	6.86	5.53
0.75% EMS	3.14	6.79	7.14	5.32	6.19	4.39	3.85	6.89	6.45	5.32	4.89	5.68	6.82	5.98	4.69	7.32	6.98	5.65	5.89	7.32	5.49

P value for treatment = 4.574**

** - Significant at 1% level.

Table 17a. Yield of Green Fodder

Treatment	Mean yield of green fodder in gram
Control	587.00
15 krad	381.60
20 krad	756.00
25 krad	425.60
0.25%	765.00
0.50%	521.00
0.75%	421.00

F value for treatment = 5.461**

** - Significant

TABLE 17b. Green Fodder Yield

Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Mean
Control	630	496	583	730	715	490	559	641	586	532	760	560	484	520	655	445	541	685	485	560	587
15 Krad	420	285	340	249	420	365	389	450	320	660	421	540	221	375	340	261	350	425	318	440	380
20 Krad	749	789	785	659	821	759	749	658	882	822	841	749	685	560	780	665	835	649	721	618	756
25 Krad	550	349	486	506	435	259	389	530	405	520	359	655	385	520	320	325	414	520	480	440	425
0.25% EMS	720	789	650	809	815	621	640	813	720	671	784	725	760	580	645	780	815	615	685	702	763
0.50% EMS	581	540	678	490	725	681	450	328	546	621	345	380	450	580	612	595	469	551	495	640	521
0.75% EMS	381	540	470	389	380	460	444	528	380	640	322	498	514	468	581	344	360	420	481	340	425

P value for treatment = 5.461**

** Significant at 1% level.

next highest value of 7.81 was recorded in 0.25% EMS treatment. The minimum leaf area was by 25 krad gamma irradiation while 0.50% and 0.75% doses of EMS were found to leave little effect on the character. However the progeny rows within the treatment did not reveal any significant difference among them.

17. Effect of treatments on Green Fodder Yield

The data on yield on green fodder is given in Table 17(a) and 17(b).

There is significant difference on the yield of green fodder in M_1V_1 generation with respect to the different treatments. The maximum yield was given by 0.25% EMS treatments followed by 20 krad gamma radiation. Minimum yield was recorded by 15 krad gamma radiation. However the difference among progeny rows within treatment is not significant. The clones 3-5, 3-9, 3-10, 3-11, 3-17, 5-4, 5-5, 5-8 and 5-17 were found to be very promising. Plant graphs of clone numbers 3-9, 3-11 and 5-17 are given on Plates XI, XII and XIII.

Plate XI Clone 3-9-A very promising variant

Plate XII Clone 3-11-A promising variant

Plate XIII Clone 5-17-A promising clone

DISCUSSION

DISCUSSION

Panicum maximum is a facultative apomict with less than 5% cross fertilisation (Brown and Emery, 1958 and Burten et al. 1973). The apomictic reproduction and vegetative propagation prevalent in this crop have imposed serious limitations in genetic improvement by conventional methods of breeding. Attempts to create variability and to evolve better types through interspecific hybridization between Panicum maximum and Panicum repens failed due to cross incompatibility (Anon, 1978). The dual method of propagation, viz., seed and slip propagation prevalent in the crop makes it an excellent material for genetic improvement through mutations. Identification of superior variants followed by vegetative multiplication can lead to immediate improvement in the level of fodder production since seed production need not be resorted to for multiplication. Genetic variation induced can directly be carried forward without deterioration through slip propagation. The present study was conducted for inducing genetic variability in Panicum maximum Jacq var. Mackuenii through mutational approach using four doses of gamma rays (15 to 30 krad) and four concentrations of EMS (0.25 to 1.00%). The results obtained through this study is discussed below.

I Germination test

From the germination test conducted on the guinea grass seeds (Panicum maximum Jacq var. Mackuenii) it appears that the viability of the seeds depends on storage time of harvested seeds. Freshly harvested seeds have to undergo a dormancy period before achieving maximum viability. The very low germination percentage of fresh seeds (0.3 per cent) recorded in this study may be due to germination inhibitors found in the lemma and palea as concluded by Smith (1970). Poor germination of freshly harvested seeds was recorded by many workers (Smith, 1970; Prasad and Gopimony, 1982). In this study it was found that germination could be increased by storage. Smith (1970) and Mayer and Poljakoff-Mayber (1975) also arrived at the same conclusion. The maximum germination of 14.85 per cent was recorded by seeds which were kept for 120 days before sowing. Similar results were obtained by Prasad and Gopimony (1982). It appears that the optimum storage period for getting maximum germination and a good crop is around 100 to 120 days.

II Effect of mutagens in M_1 generation

1. Germination of seeds

Seed germination was found to be affected by gamma radiation though the effect was not much pronounced in lower doses. Such

reduction in seed germination by gamma radiation was reported by Goud (1972) in Sorghum, Vaidhyanathan (1973) in Panicum antidotale, Polomino et al. (1979) in barley, Reddy and Smith (1975) in Sorghum, Valeva (1976) in wheat,. Ayyamperumal (1977) in ragi and Sapra et al. (1978) in Triticale. Matsumura and Mabuchi (1964) observed decreasing germination with increasing doses of radiation in several crop plants. With low doses of gamma radiation a stimulatory affect on germination is noted. This increase in the percentage of germination may be attributed to the increased activity of certain enzymes in the synthesis of auxins (Casarett, 1968). Seed germination was found to decrease drastically with increasing doses of EMS. Similar results were obtained by Goud et al. (1970) in sorghum, Vareshchagin (1974) and Rahman and Soriano (1972) in rice, Vaidhyanathan (1973) in Panicum antidotale, Raveendran (1976) and Ayyemperumal (1977) in ragi, Singh et al. (1978) in pearl millet and Gupta et al. (1977) in wheat. The reduction in germination might have been due to the toxicity of EMS. This in turn is attributable to the strong acids which forms on hydrolysis and which is seen in the treatment solution and on the inside of cells during treatment (Konzhak et al. 1965).

In the present investigation the various doses of gamma rays and EMS have produced a delay in germination. Similar delay in germination has been reported by Gaul (1967) in barley and Sree Ramulu (1970) in Sorghum. This delay can be attributed to the mitotic

impairment which disrupts resistance in seeds (Cherry and Hageman, 1961). The delay in germination was found to increase with increase in dose of mutagen. Shirshov and Shain (1966) reported similar results using gamma rays in field beans. Also it is seen that EMS treated population recorded pronounced delay in germination compared to irradiated ones. This effect of EMS in delaying seed germination is clearly demonstrated by Sree Ramulu (1970) in rice and Chandrasekhar and Reddi (1971) in Sorghum.

Physiological effect of mutagens in inhibiting germination was reported by Chauhan and Singh (1975). According to them gamma rays cause disruption and disorganisation in the tunical layers which result in poor germination of exposed seeds. A most striking effect is the impairment of mitosis and virtual elimination of cell divisions in meristematic zone during germination of seeds as reported by Cherry and Hageman (1961) in corn. Influence of mutagens in germination was attributed by Skoog (1935) and Smith and Kerstan (1940) to the destruction of auxins while Gordon and Webber (1955) and .Gordon (1957) suggested that it could be due to inhibition of synthesis of auxins. Chemical mutagens especially alkylating agents are known to react with DNA by alkylating phosphate groups (Alexander and Stacey 1958). Inhibition of germination with EMS treatment was found to be due to formation of acids upon hydrolysis which in turn reduce pH of media making it toxic (Freeze Gertzen et al. 1963).

2. Survival of Seedlings/plants

Percentage of plants surviving in the treated population determines post germination lethality. Survival is one of the factors which determines the largest possible exposure of a mutagen that can be applied and used as a relative measure of effect of exposure employed. In this study the survival percentage was found to decrease with increase of dose of gamma rays. Similar observations were reported by Sapra et al. (1978) in Triticale, Choudhury (1978) in wheat, Raveendran (1976) in ragi, Singh (1971) in rice and Gottschalk (1967) and Ramulu (1974) in sorghum. The reduction in survival is an index of post germination mortality in treated plants as a result of cytological and physiological disturbances due to radiation effect. The cytological abnormalities caused by irradiation may lead to structural changes in the chromosomes (Ehrenberg et al. 1959). This interferes with the normal growth and development of organs which might have led to the fall in survival percentage with irradiation. Konzak et al. (1965) attributed the decrease in survival percentage with increasing doses of radiation to the reduced cell growth resulting from cytological abnormalities and also due to decrease in the synthesis of auxins and other physiological change. This ultimately results in the poor growth and development of organs and reduction in survival percentage with increased doses.

In EMS treatment the higher doses have a deleterious effect on survival of seedlings. A drastic reduction in survival percentage is noted with increase in doses of EMS. This is probabily because of poor germination caused by the higher doses and not per se on survival of germinated seedlings. Similar results were obtained by L'vova and Konorovskaya (1974) in barley and Singh et al. (1978) in pearl millet and Vijayagopal and Nair (1985) in rice.

3. Plant growth

A reduction in plant height as a result of gamma ray treatment was noted in certain doses of the mutagen in the present investigation. Woodstock and Justice (1967) after studies in maize, wheat and sorghum have reported a proportional decrease in growth rate depending on increase to exposure load of gamma rays. Reduction in plant height following gamma irradiation was reported by El-Aishey et al. (1976) and Kapoor and Natarajan (1970) in wheat, Goud et al. (1969) and Ayyamperumal (1977) in ragi, Sree Ramulu (1974) in sorghum, Vijendra Das (1978) in bajra and Geilo and Starzycki (1978) in Triticale and Vaidyanathan (1973) in Panicum antidotale.

Both internal metabolism and external conditions bear a direct or indirect influence on growth of plants. Ehrenberg (1960) has reported a toxic chemical which influences plant growth. Irradiation stops DNA transcription and leads to reduction in protein synthesis and that reduction of growth in irradiated meristem is the cumulative expression

of three types of cytologically identifiable effects viz., (1) mitotic delay (2) formation of chromosomal abberations (3) loss of proliferative capacity of cells due to premature differentiation or death of cells (Evans , 1965). Later on, it was found that first two factors had only minor roles in bringing about growth depression and it was concluded that the major cause was due to loss of proliferative capacity of cell.

Evans and Sparrow (1961) believed that the influence of ionizing radiation on growth can be attributed basically to gene loss due to chromosomal abberations, Pollard (1964) postulated that irradiation inhibits the transciption of DNA and leads to a decrease in messenger RNA which should cause a decrease in protein synthesis and growth. Conger et al. (1969) after exposing barley seeds to radiation found that damage to plant height and to chromosomes are closely correlated even within a treatment.

Reduction in plant height was also seen in EMS treatments. Similar reductions in plant height was reported by Soriano (1968) in rice, Sharma (1970) in barley and Singh et al. (1978) in pearl millet. Ramulu in sorghum and Ayyamparumal (1977) in ragi reported gradual decrease in the mean of plant height as the concentration of chemical mutagens increased.

From this study it is seen that certain lower doses of gamma radiation and EMS shows a stimulatery effect on height compared to control plants. Similar results were reported by Gunckel and Sparrow

(1961) and Sax (1963) and Ganuscvichyute (1971). Increase in seedling growth at lower doses of mutagen treatment can be due to destruction of inhibitory substances and an increase in physiologically active substances like auxin, gibberellin etc. which stimulate elongation.

III M_1V_1 Clonal progeny

Vegetatively propagated plants which are amenable to seed propagation also, are at a distinctively advantageous position for crop improvement, through mutation breeding. Vegetatively propagated plants usually preserve all the heterozygosity through generations. Heterozygosity in the irradiated material makes possible the detection of induced mutations in the M_1 generation itself. Most mutations being recessive, can be easily detected in the M_1 generation of the heterozygous plants. Vegetative propagation of such M_1 plants into M_1V_1 clonal progeny and a critical evaluation of their performance compared to control progeny can facilitate isolation of superior clones. Nair (1979) successfully isolated many superior clones of lemongrass from the M_1V_1 progeny obtained from the M_1 chimeric plants through gamma irradiation. M_1V_1 generation in the present study did not reveal any significant difference in the tillering habit for the different treatments. None of the clones were also found to be superior to control clones with respect to number of tillers. Contrary to the findings of Gupta and Athwal (1966) the higher fodder yield did not appear to have been influenced by high tillering. The treatments also did not

appear to influence height of plants. Therefore the increased green fodder yield was not influenced by this character. The effect of treatments on girth of internode and leaf area index were significant. While the clones within the treatment revealed significant difference for the girth of internode, the superior clones of the superior treatments were also significantly different from control clones.

A comparison of effects of treatments in the M_1V_1 generation revealed that the progenies from treatments 20 krad gamma radiation and 0.25% EMS, had yields significantly above the control progenies. However the clones within the treatments did not reveal significant difference in their green fodder yield. The clones 3-5, 3-9, 3-10, 3-11 and 3-17 from 20 krad gamme irradiation and 5-4, 5-5 and 5-8 and 5-17 from 0.25% EMS were significantly superior to the control clones. These clones were identified to have superior leaf area index and higher girth of internode. None of the other clones in other treatments proved superior to the control clones. Guptha and Athwal (1968) and Guptha (1968) were of opinion that high fodder yield was due to more leafiness. The present study also reveal that the enhanced fodder yield appear to be due to increased leafiness as indicated by the higher leaf area index and girth of internode.

SUMMARY

SUMMARY

Guinea grass being a facultative apomict under continuous vegetative propagation exhibits very little variability. The objective of the study was to induce variability through induction of mutations in the cultivar Makuenii of grass species Panicum maximum. Seeds were subjected to mutagen treatments and M_1 and M_1V_1 generations were studied.

The mutagens used were gamma radiation at four doses ranging from 15 krad to 30 krad with increments of 5 krad and the chemical mutagen, EMS at four concentrations from 0.25 per cent to 1.00 per cent with increments of 0.25 per cent.

The following observations were recorded in the M_1 generation.

Freshly harvested seeds of guinea grass exhibited dormancy for quite a long period and the maximum viability of 14.85 per cent was obtained 120 days after harvest. When such seeds were subjected to mutagen treatment, the germination of seeds was found to be affected. The reduction in germination was observed to increase with increase in doses of mutagen. EMS exhibited greater inhibitory effect on seed germination than gamma rays.

Both the mutagens had deleterious effect on survival of seedlings. While the initial germination was poor after chemical treatments compared to gamma irradiation, a good proportion of germinated seed

survived in chemical treatments unlike in the gamma ray treatments which showed considerable post germination mortality.

The growth of seedlings was affected by the treatments with both the mutagens, but, however, the survived seedlings appeared to recover from the shock of the treatment and the growth approached normality in the later stages of their growth.

The effect of mutagens on the survival of plants upto flowering indicated that there was considerable mortality of seedlings immediately after transplantation. However at later stages, the effect of gamma radiation was more pronounced than that of EMS.

The highest doses of mutagens caused the greatest reduction in plant height while the lower doses did not appear to have any effect on the growth of plants.

The mutagen treatment did not appear to have significant effect on the production of tillers. However, the maximum tiller count was recorded by the control plants.

The higher doses of mutagens reduced flower initiation as indicated by the reduced number of inflorescence. Between EMS and gamma rays, the chemical treatment showed more inhibitive effect on flowering.

Chlorophyll chimeras were observed in the M_1 generation. The lower doses seemed to record more number of chimeras probably because of the elimination of mutations at higher doses. Plants with various morphological abnormalities were also noticed in the M_1 generation.

Tiller counts made in the M_1V_1 generation revealed that the treatment did not have any effect on tiller numbers. The clones within each treatment also did not exhibit variation.

The means of the M_1V_1 clones showed variation with the control means, in respect of plant height. While the chemicals reduced the plant height, the 10 krad and 20 krad gamma ray treatments caused a positive shift in the treatment means.

A study of variations in the girth of internode indicated a positive shift in the treatment means, of 20 krad gamma rays and 0.25 per cent EMS from control means.

The treatments 20 krad gamma rays and 0.25 per cent EMS had higher means for leaf area index, compared to control in the M_1V_1 germination.

The clones 3-5, 3-9, 3-10, 3-11, 3-17, 5-4, 5-5, 5-8 and 5-17 were identified to have higher fodder yield than that of the control. These clones also had higher leaf area index and higher girth at internode suggesting that the higher green fodder yield might be due to the increased leaf area and higher girth of internode.

The study revealed that variability with respect to height, girth of internode, leaf area index and green fodder yield can be induced by mutations using appropriate doses of gamma rays and EMS. The medium dose of 20 krad gamma rays and a relatively low concentration of 0.25 per cent EMS appeared to be useful to induce variations in guinea grass.

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* Originals not seen.

APPENDICES

ABSTRACT OF ANALYSIS OF VARIANCE TABLES

APPENDIX I

Sl. No.	Characters studied	Mean square		F
		Treatment (8)	Error (18)	
1.	Germination			
a)	5th day	112.97	14.93	7.56**
b)	6th day	101.11	21.24	4.76**
c)	7th day	161.48	43.39	3.72**
d)	8th day	246.19	81.89	3.01*
e)	9th day	283.78	47.53	5.97**
f)	10th day	331.02	47.61	6.95**
g)	20th day	79.02	52.25	1.48
h)	30th day	74.85	46.85	1.59
2.	Survival of seedlings			
a)	1st week	276.38	51.67	5.35**
b)	2nd week	281.34	50.47	4.96**
c)	3rd week	224.32	52.04	4.13**
d)	4th week	206.18	41.84	5.12**
e)	5th week	190.32	32.86	5.69**
3.	Seedling height			
a)	2nd week	2.84	0.70	4.06**
b)	3rd week	16.63	2.59	6.39**
e)	4th week	121.45	5.39	22.53**
f)	5th week	144.56	32.91	4.29**

* Significant at 0.5 level

** Significant at 0.01 level

APPENDIX II

Sl. No.	Characters studied	Mean Square			F
		Block (2)	Treatment (8)	Error (16)	
1.	Survival of plants				
	a) 1st Month	23.27	713.83	218.07	3.47*
	b) 2nd Month	251.58	607.00	114.54	5.30**
2.	Height of plants				
	a) 1st observation	261.791	34.64	225.16	0.15
	b) 2nd observation	185.23	2102.9	525.85	3.99**
3.	Tiller Counts				
	a) 1st observation	28.57	4.23	10.31	0.41
	b) 2nd observation	14.10	44.43	20.40	2.17
4.	No of inflorescence per clump				
	a) 1st observation	26.79	34.64	225.16	0.15
	b) 2nd observation	2102.90	185.24	525.86	0.35
5.	Pollen sterility	630.19	601.21	183.54	3.86**

* Significant at 0.05 level

** Significant at 0.01 level

INDUCTION OF GENETIC VARIABILITY IN GUINEA GRASS (*Panicum maximum* Jacq.) Var. Makuenii

By

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ABSTRACT OF THE THESIS
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ABSTRACT

Guinea grass (Panicum maximum J.) being a facultative apomict under continuous vegetative propagation carry very little variability. The cross incompatibility also restricts attempts to produce variability through conventional methods of plant breeding. The main objective of the study is to induce variability through mutations in guinea grass variety Makuenii using gamma rays at four doses ranging from 15 Krad to 30 Krad and EMS at four concentrations ranging from 0.25 percent to 1.00 percent. The effect of the mutagens in M_1 and M_1V_1 generations were studied.

The observations in the M_1 generation indicated that germination of seeds was progressively reduced with increase in the dose of mutagens. The survival and early growth of seedlings were adversely affected by the mutagen treatments. The seedlings appeared to suffer from the transplanting shock as indicated by high mortality of the plants in the field. Higher doses of mutagens induced height reduction. However the tillering ability of the plants did not seem to have been affected but higher doses of mutagens inhibited flowering initiation. Pollen sterility in M_1 generation exhibited dose dependance. A few chlorophyll chimeras were observed in the M_1 generation. Morphological abnormalities induced included dwarf, tall, semi open and open types with leaf and inflorescence modifications.

The means of M_1V_1 clones exhibited both positive and negative shifts from the population means with respect to plant height. Treatments 20 Krad gamma rays and 0.25% EMS caused positive shifts in

the means of girth at internode and leaf area index. Certain clones were identified to have higher green fodder yield than the control. These clones also had higher girth at internode and higher leaf area index.

The study revealed that variability with respect to plant height, girth at internode, leaf area index and green fodder yield can be induced by mutations using appropriate doses of gamma rays and EMS. A medium dose of 20 krad gamma rays and a relatively lower dose of 0.25 percent EMS were found to induce more useful variations.