STUDIES ON THE EFFECT OF LASER RADIATION AND OTHER MUTAGENS ON PLANTS

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by

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Dedicated to

My family members

and

to the memory of late J.V. Pillai (1946-1993)

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CERTIFICATE

This is to certify that the work presented in the thesis entitled " **STUDIES ON THE EFFECT OF LASER RADIATION AND OTHER MUTAGENS ON PLANTS**" is based on the original research done by Mr. P.R. Unnikrishna Pillai, under my guidance and supervision, at the Department of Biotechnology and no part of the work has been included in any other thesis for the award of any degree.

P. Nambisan

Padma Nambisan

DECLARATION

I hereby declare that the work presented in the thesis entitled "**STUDIES ON THE EFFECT OF LASER RADIATION AND OTHER MUTAGENS ON PLANTS**" is based on the original research work carried out by me at the Department of Biotechnology, Cochin University of Science and Technology, under the guidance of Dr. Padma Nambisan, Lecturer (Plant Biotechnology) and no part thereof has been presented for the award of any other degree.

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CONTENTS

ABSTRACT

The effect of lasers of three wavelengths in the visible region - 476, 488 and 514 nm on mitotic and meiotic cell divisions, growth, yield and activity of specific enzymes were studied in two taxonomically diverse plant species - *Allium cepa* L. and *Vicia faba*. The effect of laser exposures was compared with the effect of two physical mutagens (Gamma and Ultraviolet radiations) and two chemical mutagens (Ethyl Methane Sulphonate and Hydroxyl amine). The study indicated that lasers could be mutagenic causing aberration in the mitotic and meiotic cell divisions while also producing changes in the growth and yield of the plants. Lasers of higher wavelengths 488 and 514 nm caused aberrations in the early stages of mitotic cell division whereas lasers of lower wavelengths (476 nm) caused more aberrations in the later stages of mitotic cell division. Laser exposure of 488 nm wavelength at power density 400 mW induced higher mitotic and meiotic aberrations and also induced higher pollen sterility than lasers of 476 and 514 nm. The frequency of mitotic aberrations induced by lasers was lesser than that caused by γ-irradiation but comparable to that induced by EMS and HA. Lasers cause mutations in higher frequencies than UV. Lasers had a stimulatory effect on growth and yield in both plant species. This stimulatory effect of lasers on germination could not however be correlated to the activity of amylase and protease, the key enzymes in seed germination. Enzymes such as peroxidase and catalase, involved in scavenging of free oxygen radicals often produced by irradiation, did not show increased activity in laser irradiated samples. Further studies are required for elucidating the exact mechanisms by which lasers cause mutations.

INTRODUCTION

Hugo de Vries, the Dutch botanist, in 1900 coined the term 'mutation' for sudden hereditary changes in *Oenothera lamarckiana*, the evening primrose. But mutations were known to occur in animals and plants much before this time. For example, Seth Wright, an English farmer discovered a short legged sheep in 1791; this sheep was used to establish a breed named Ancon. Mutations may be chromosomal, cytoplasmic or gene mutations (point mutations). Mutations may occur spontaneously at a low frequency of one in 10 lacs i.e., 10^{-6}, are usually recessive, deleterious in effect, random and recurrent in occurrence. Induced mutagenesis provides a base for strengthening crop improvement programmes by generation of genetic variability.

1.1 History of Mutation Research

Auerbach (1976) divided the history of mutation research into five periods. The first period was between 1900 to 1927. During this period the concepts of mutation and mutation rates were developed, the basic questions about the nature of mutation were formulated and techniques for measuring mutation rates were worked out. The discovery of the mutagenic action of X-rays in 1927 lead to the starting of the second period. X-rays provided a tool uniquely suitable for probing into the nature of mutation. The 'target theory' an unified theory of mutation had been constructed by the end of the second period in the late thirties. The target theory was that the genes and the presumed links between them are 'targets' for 'hits' by energy packets that are delivered to them by ionizing radiation. Point mutation is produced by a hit in a gene and a chromosome break by a hit in a link between genes. Just before the

second world war, the third period of mutation research started. At that time chemical mutagens became available. Also micro-organisms were introduced as a means of experimental analysis for studying the mutagenic action especially by ultraviolet radiation. A chemical model of the gene was necessary for interpretation of most of the findings of this period. Such a model was however not available till 1953 when Watson and Crick provided a structural model for DNA. The fourth period, from 1953 to about 1965, was dominated by studies on the chemistry of DNA and the action of mutagens on DNA. It was later realised that cellular mechanisms such as repair and expression, play important roles in the mutation process. In the present fifth period which started in 1965, the physical and chemical knowledge obtained during the preceeding ones is being used as the basis for analysing mutation as a biological process.

In the early part of the 20th century, experiments conducted in Morgan's laboratory using mutants of *Drosophila* lead to the realisation that the nature of mutation was inextricably linked with the nature of the gene. In 1927, H.J. Muller presented data at the Third International Congress of Genetics that demonstrated unambiguously the capacity of X-rays to produce mutations. The following year, L.J. Stadler demonstrated the same in maize. Subsequently X-ray mutagenesis dominated almost all branches of genetics for two decades. That UV could produce mutations in *Drosophila* and flowering plants had been established by the early thirties. Ionizing radiations such as Gamma were used for the production of mutations in agricultural plants from the end of 1930's. In 1946, Auerbach and Robson demonstrated that mutations could be produced by nitrogen mustards in *Drosophila*. Although this was the first application of a chemical mutagen, several others became available later on and found widespread application in inducing mutations.

1.2 Nature of Mutations

Mutations are of different types. Mutations produced by changes in base sequence of genes as a result of base pair transition or transversion, deletion, duplication or inversion etc. are known as gene mutations. Those produced by changes in chromosome structure or even in number are termed as chromosomal mutations. Gross chromosomal changes such as changes in chromosome number, translocations, inversions, large deletions and duplications are detectable under the microscope. In cytoplasmic mutation, the mutant character shows cytoplasmic or extranuclear inheritance. Bud mutations or somatic mutations occur in buds or somatic tissues which are used for propagation, e.g. in clonal crops.

New alleles are rarely produced in induced mutations but they produce alleles which are already known to occur spontaneously or may be discovered if an extensive search is made. The effects and the variability produced by induced and spontaneous mutations are comparable. The great advantage of induced mutations over the spontaneous ones is that they occur at a relatively higher frequency so that it is practical to work with them. Mutations have certain general characteristics which are summarised below :

1. They are generally recessive, but dominant mutations also occur.
2. A small portion (0.1%) are beneficial but a majority of mutations are generally harmful to the organism.
3. Mutations may occur at random in any gene. However, some genes show higher mutation rates than the others.
4. The same mutation may occur again and again, that is, they are recurrent.

1.3 Effects of Mutation

In general, mutations have harmful effects on organisms. The viability of the individuals that carry mutations is usually reduced. Mutations are classified into four groups, based on their viability. They are :

Lethal mutations : This type of mutation normally kills all individuals that carry them. Dominant lethal mutations affect even heterozygous individuals, while recessive lethals kill only the individuals which carry them in the homozygous state.

Sub lethal and Sub vital mutations : Do not kill all the individuals that carry them but reduce the viability. Sublethals kill more than 50% of the individuals while subvitals kill much less than 50%. This type of mutations are of no value although a vast majority are of these type.

Vital mutations : The viability of the individuals carrying this type of mutation is not reduced. This class of mutations occur in a much lower frequency than the other three types, but are the only ones that can be utilised in crop improvement.

1.4 Induction of mutations

Treatments with certain agents known as mutagens can be used to induce mutations at relatively higher frequencies. Mutagens may be different kinds of radiations (physical mutagens) or certain chemicals (chemical mutagens).

1.4.1 Chemical Mutagens

These are broadly classified into:

1. Alkylating agents, for instance

   Sulphur mustards

   Nitrogen mustards

   Epoxides

   Ethylene imines (EI) e.g. Ethylene imine

   Sulphates and Suphonates, e.g. Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS)

   Diazoalkanes

   Nitroso Compounds, eg. N'-methyl-N-Nitro-N-nitroso-gunanidine (MMNG)

2. Acridine dyes, eg. acriflavine, proflavine acridine orange, acridine yellow, ethidium bromide
3. Base analogues, eg. 5-Bromouracil, 5-Chlorouracil
4. Others, eg. nitrous acid, hydroxyl amine, sodium azide

Mechanism of action of chemical mutagens

The mechanism of action of each group of chemical mutagens is different. A mutagen may produce mutagenic or inactivating changes in DNA. While a mutagenic change does not prevent replication, it produces changes in one or more nucleotides and does not induce chromosomal aberration. Such changes are the results of changes in hydrogen bonding properties of bases or mistakes in base pairing during DNA replication. Inactivating alterations prevent DNA replication across the altered site, induce chromosome breaks and chromosome mutations. The inactivating alterations of DNA in most of the cases are repaired by cellular enzymatic repair mechanisms.

1.4.2 Physical Mutagens

These include various kinds of radiations. Such as

1. Ionising radiations
    a. Particulate radiations
        α-rays (Densely ionizing)
        β-rays (Sparsely ionizing)
        Fast neutrons (Densely ionizing)
        Thermal neutrons (Densely ionizing)
    b. Non particulate radiations (Electro magnetic radiations)
        X-rays (SI)
        γ -rays (SI)
2. Non ionizing radiations
        UV radiation

X-rays and γ-rays

X-rays and γ-rays are similar in physical properties and biological effects but they differ in the source of their origin. X-ray tubes are used to produce X-rays while radioactive decay of certain elements produce γ-rays, e.g., radium, ^{14}C, ^{60}CO etc. The common source of γ-rays used for biological studies is ^{60}CO. Depending upon the wavelengths, X-rays are often referred to as hard or soft. X-rays are high energy radiations (small packets of energy) and are sparsely ionising. They produce Photoelectric Effects, Compton Scattering and Pair Production.

UV radiation

UV is a low energy radiation, does not cause ionisation and has a very limited penetrating capacity (one or two cell layers). It is present in solar

radiation and can also be produced artificially by Mercury vapour lamps or tubes. Generally, UV rays produce dimers of thymine, uracil and cytosine present in the same strand of DNA. It also produces addition of a molecule of water to the 5,6 double bond of uracil and cytosine, which promotes deamination of cytosine. The dimer formation and deamination are likely to be the reason for the mutagenic action of UV. In micro-organisms, UV is commonly used to induce mutations since penetration presents no problem in that system. Use of UV in higher organisms is limited to irradiation of pollen grains (in plants) and to small eggs (e.g. in *Drosophila*).

Mechanism of action of radiations

Radiations are direct as well as indirect in their effects. Energy is transferred directly by the radiation to a molecule in direct effect but in indirect effect it is mediated by free radical formation : the radicals transfer their energy to other molecules. The indirect effect is particularly important in presence of water since ionised water molecules produce free radicals (Singh, 1983).

According to Evans (1962) radiation does not produce direct breakage in chromosomes, but initiates a lesion requiring DNA synthesis for repair. An exchange would arise as a consequence of misrepair of the lesions. Revell (1959) have stated that all aberration are a consequence of exchange following a process of misrepair of primary lesions. Variations in radio sensitivity at different stages of the cell cycle are due to differences in the time available for repair and to changes in chromatid structure during chromosomal replication.

Radiations have been extensively utilised for many years to cause mutations and chromosomal damage for experimental purposes. They can induce a change in the molecular organisation of protoplasm. The change may be expressed as a mutation, a break in a chromosome, or an alternation in the physiological activity of the cell (Cohn, 1969). The manner in which the yield of structural changes increases with increase of the dose of radiation has been extensively studied, and the results of these studies form the main basis on which theories of the mechanism of induction of these changes are built (Lea, 1946).

1.5 Effect of light in the visible range on plants

A number of processes in plants such as photosynthesis, protoplasmic streaming, flower induction, seed germination, chlorophyll biogenesis, bending of organs and numerous other growth reactions are affected or controlled by radiant energy. Under optimum conditions these processes are normal. But variations above a certain range induces stress symptoms in plant (Noggle and Fritz, 1983). Radiation between 510 and 610 nm (green yellow) has minimal effect on plant growth where as radiation between 400 and 510 nm (blue) will have the following effects - chlorophyll and other photosynthetic pigments such as phycocyanin, phycoerythrin and carotein have peaks in absorption in these wavelengths. Phototropic movements of plants are promoted by absorption of radiant energy of these wavelengths. It has been thought that visible radiation will not be mutagenic since most of the biological molecules have optical absorption in the UV region. Since the discovery of laser in 1960, the idea that the visible light may be mutagenic has been mooted. Putative mutagenicity has been attributed to the coherence and

intensity of laser beams, due to which it can interact with biomolecules in a non-linear way.

Laser is an acronym for Light Amplification by Stimulated Emission of Radiation. Laser beam consists of photons having associated wavelengths that are exactly in phase, hence the term coherent. Laser beam is highly directional and due to this property it is extremely bright i.e., power per unit area per solid angle is very high and it is monochromatic. Lasers are produced by exciting an absorbing material with electromagnetic radiations. Lasers are classified as gas lasers, dye lasers, semiconductor lasers, etc. In the case of gas lasers, pumping is caused by passing an electric current. Examples for gas lasers include Helium-neon laser, Argon ion laser, Krypton ion laser etc. There are infrared, visible and ultraviolet lasers. Visible lasers normally function in wavelengths between 400-600 nm.

Modern laser techniques provide a wide range of variation of radiation parameters such as frequency, intensity and pulse duration thus making it possible to carry out investigations on selective action on substances. Molecules or part of molecules of the same type may undergo considerable change caused by photoionisation or photodissociation with subsequent chemical reactions.

Objectives of the present study

Genetic improvement of crops is dependent on the availability of genetic variability. Sources to induce such variability include the use of physical and chemical mutagens, tissue culture etc. Although lasers have recently been suggested to be useful in inducing mutations, their use has been limited in the

absence of any systematic study to establish their precise mutagenic nature. In recognition of this, the specific objectives of the present study were as follows :

1. To study the effect of laser radiation in inducing chromosomal aberration during mitotic cell division by analysing the root tip squashes of two plant species - *Vicia faba* and *A. cepa* L. in comparison to the effect of physical mutagens such as γ-rays and UV -rays, and chemical mutagens such as Ethyl Methane Sulphonate (EMS) and Hydroxyl Amine (HA).
2. To compare the effect of laser radiation and the aforesaid mutagens on mitotic index in the two plant species.
3. To study the effect of laser radiation on meiotic cell division in the flower buds of *V. faba* in comparison to the effect of the other mutagens.
4. To compare the pollen grain sterility in these different treatments.
5. To study the effect of laser radiation and the other mutagens on growth and yield in the two plants species.
6. To study the effect of laser irradiation on enzyme activity, specific activity and soluble protein content in comparison to the effect of EMS and γ-irradiation.

REVIEW OF LITERATURE

2.1 Mutation research in plant breeding

In mutation breeding, mutants are produced by inducing variations using either chemical or physical mutagens. The methods of experimental mutation research are utilised in plant breeding since about 40 years. Induced mutations in crop plants contribute by increasing genetic variability.

Effective treatments are essential for economical use of mutagens as tools for the induction of heritable changes in qualitative and quantitative characters of crop plants. A number of useful varieties of food crops and ornamentals have been developed by making use of mutations (Micke, 1961). Both epigenetic and genetic changes have been found to be useful in plant improvement. Epigenetic changes increasing growth, yield or secondary metabolite production in vegetatively propagated crops and genetic mutations affecting increased yield, stress tolerance, disease resistance, protein quantity or quality, etc. are of use in crop plants.

2.2 Mutagenic effectiveness and efficiency

"Effectiveness" is a measure of gene mutation in relation to dose and "efficiency" is an estimate of biological effects induced such as, lethality, injury and sterility. (Konzak *et al.* 1965). According to Blixt (1968), the sensitivity of an organism depends up on the mutagen employed and its genetic make up. The usefulness of any mutagen depends upon not only its effectiveness but also to a large extent upon its efficiency. Effective

mutagenesis is brought about by the production of useful mutation with minimum undesirable changes.

The different response of varieties to different mutagens have been reported by Prasad and Das (1980), Bhamburkar and Bhalla (1980) and Karpate (1996). The type of mutagens, plant genotype and the physical state of the organism are considered to be important factors which contribute to the difference in frequency and spectrum of induced mutations (Prasad and Das, 1980; Bhamburkar and Bhalla, 1980; Reddy *et al.*, 1993). The difference in mode of action of mutagen (Okado *et al.*, 1972), differential penetration of the mutagen to the target (Kihlman, 1952), efficiency of repair process (Lawrence *et al.*, 1974) and factors affecting the expression of concerned mutation (Auerbach, 1967) might also be playing a role in inducing mutations with varying frequency and spectrum. Sharma and Chatterjee (1962) and Varugheese and Swaminathan (1968) are of the opinion that the difference in mutagenic effectiveness and efficiency are due to the amount of DNA and its replication time in the initial stages. It might be due to the physiological stage of the cell, ability to repair the damage or several other physical factors (Brock 1965; Chopra and Swaminathan, 1966; Auerbach, 1967; Gelin, 1968; Ilivea, 1971).

Index in determining the biological effects of various mutagens

Germination, survival and seedling growth are widely used as indices in determining the biological effects of various mutagens. The reduction of these parameters were prominent in EMS treatments either alone or in combination. Such an inhibitory effect of various mutagens was reported in several crops (Reddy *et al.*, 1991 a; 1992; Reddy and Aloka saika, 1992;

Pushpalatha *et al.*, 1992). Reduction of these parameters has been attributed to various factors including changes in the balance of growth regulators and metabolic activity (Aman, 1968), physiological changes including inhibition of DNA synthesis (Gordon, 1957), or inhibition of mitotic proliferation (Sparrow, 1961). Increase in seedling injury could be due to mitotic irregularities (Sharma and Govil, 1986) Sterility in pollen is mainly due to interchanges between non-homologous chromosomes and detectable chromosomal aberration (Suganthi and Reddy, 1992).

2.4 Biochemical effects and dose dependence of mutagens

Mutagens affect the metabolism of the individuals and influence the activity or synthesis of enzyme and growth regulators (Jain and Khanna, 1987). Such harmful effects of mutagens lead to various forms of physiological expression of damage such as retarded plant growth, induction of mutations, sterility and death.

Inhibition of germination after mutagenic treatments has been attributed to chromosome organisation (Evans and Sparrow, 1961), and changes in variety of biochemical and physiological systems (Sparrow and Woodwell, 1962). The depression in the rate of mitotic proliferations may be the cause of delay in seed germination (Favret, 1963). The denatured DNA after sometime may be repaired resulting in the activation of biological process involved in germination and thus the germination may also be delayed (Hutterman *et al.*, 1978)

Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances

Gordon, 1957; Gunkel and Sparrow, 1961; Singh, 1974 ; Usuf and Nair, 1974). Chromosome breakage during mitotic inhibition (Evans and Sparrow, 1961) has also been implicated as causes of reduced plant growth.

Mutations affecting the plant height have been reported by several workers (Chen and Gottschalk, 1970; Oknno and Kawai, 1978; Raisinghami and Mahna, 1994; Prasad and Ramesh, 1996). The mutants affecting the plant height in many crops was found to be due to changes in the internodal length and sometimes because of alternations in number of nodes (Weber and Gottschalk, 1973). Moreover, the internodal length was found to be affected by cell number, cell length or both (Bloustein and Gale, 1984).

Low doses of radiations have been found to have a stimulatory effect in different crops (Sparrow, 1966; Khanna, 1988). Stimulation of growth observed at lower doses might be caused by elevated auxin level (Gordon, 1957; Gowda, 1977). A similar response was noticed in Rhodes grass by Krishna *et al.*, (1984) and in peanut and cherry by Ashri and Herzog (1972).

In corn seedlings, Evans and Sparrow (1961) noted that the production of proteins, soluble nucleotides and RNA was reduced by X-irradiation and indicated that this reduction was roughly parallel to growth reduction). Similar results were also observed in peanut by Van Huystee *et al.*, 1968). Both DNA and RNA contents were increased in lower dose and decreased in higher dose in cowpea (Khanna, 1991). Venkatachalam and Jayabalan (1995) reported that the mean values of plant height and contents of chlorophyll, free amino acids, proteins and nucleic acids were increased upto LD 50 dose / concentration, there after, they were slightly decreased than of the control in groundnut. This may be due to the saturation effect of mutagens.

Similar observations have been made in rice (Inoue *et al.*, 1975), in chickpea (Khanna, 1991), and in barley seedlings by (Giacomelli *et al.*, 1967).

2.5 Chromosomal aberration studies

One of the oldest, simplest and least expensive methods for studying the induction of chromosomal aberration utilises plant root tips as experimental material. Of all the plants, where root tip mitosis has been studied for induced chromosomal aberration, only few, for instance *Vicia faba, A. cepa, A. proliferum, A. fistulosum* have proved to be favourable materials (Evans, 1962., Kihlman, 1966, 1975., Rieger and Michaelis, 1962, 1967). The sutability of these plants for cytological studies has been attributed to their large chromosomes, low chromosome number, supplemented by easier cultivation and availability throughout the year. The merits of these materials were realised by radiobiologists in 1930's (Khilman, 1975). Several types of studies have been done in this field so far for instance, Kihlman *et al.*, (1977) have studied the molecular mechanism in the production of chromosomal aberration with the 5 - Bromo deoxyuridine labelling method in *V. faba* while Anderson and Kihlman (1987), studied the localisation of chemically induced chromosomal aberration in three different karyotypes of *V. faba*.

2.6.1 Cytogenetics and Mutational effects of laser radiation

Laser irradiation with the wavelength equal to 337 nm on *Allium fistulosum* and *Hordeum vulgare* cells causes an appearance of chromosome aberration. In the presence of chromophores, the cytogenetic effect may be explained by direct effect of laser irradiation on chromatin DNA. Such a possibility has been demonstrated in experiments with pBR 322 DNA in the presence of ethidium bromide and riboflavin. Chromophores absorb the energy

of laser irradiation according to two-quantum mechanism. The following energy migration from donor (chromosphore) to acceptor (DNA) produces breakage of phosphodiester bonds (Dragan and Khrapunov, 1993). Vasileva *et al.,* (1991) carried out investigations for the purpose of assessing the mutagenic effect of laser irradiation of cvs Auralia and Doukat. The treated seeds were dry, soaked in tap water (15-18 hours) or soaked in a solution of the stain Rhodamine B (RhB) pea seeds. Various doses of helium neon laser (lambda - 631.8 nm) and argon laser (lamda -457.9, 488 and 514 nm) were applied. The experimental data showed cytogenetic effect of laser irradiation depending on doses, wavelength, metabolic state and cultivar of the seeds. Dry seed irradiation of cv. Auralia with helium-neon laser produced higher percentage of mutation changes at doses 0.43 and 1.72 J/cm^{-2}. The spectrum was wider at does 1.28 and 1.72 J/cm^{-2}. Irradiation effect was higher on seeds soaked in tap water and highest in seeds soaked in the RhB stain. Mutation frequency increased with the rise of dose and the spectrum was wider at doses 0.86 and 1.28 J/cm^{-2}. In cv. Doukat the effect of helium - neon irradiation was slightly expressed. Argon laser irradiation with lambda - 488 nm produced higher effect on cv. Auralia dry seeds at the higher doses 20 and 26.74 J/cm^{-2}. cv. Auralia proved more sensitive to this treatment and manifested higher mutability. Electrophoretic analysis of peroxidase showed that cv. Auralia reacted faster to the applied irradiation (lambda 488 nm).

Akhmedova (1993) conducted a study to determine the laser irradiation effect on *Gossypium* seeds. Presowing irradiation of seeds had a stimulating effect on M1 plants. The subsequent generations revealed a whole range of mutations typical of *Gossypium*. The number of mutants depended on the irradiation intensity and some of them can be used for breeding.

Xu Meiren (1991), used the methods of isoelectrofocussing polyacrylamide gel electrophoretic and micronucleus technique in the study to inspect the mutagenic effect of three lasers on L_1 wheat. During the division interphase of the pollen mother cells, the frequency of the micronucleated cells of the plants irradiated by lasers were 0.13 - 0.19% higher than that of the control, those irradiated by $15^{W}CO_2$ laser for 1 min displayed the highest. The study also showed that the lasers caused variation of characters of L_1 an L_2 wheat. The radiation of the $15^{W}CO_2$ laser for 2 min was the most effective.

2.6.2 Effect of lasers on germination, biomass and metabolic activities

Influence of laser beam of three different wavelengths - 337.1 nm, 510 nm and 632.8 nm on germinating maize seeds was carried out to study some metabolic process in seedlings (Kerpesi *et al.*, 1992). The results showed that during the period of investigation (1-6 days), the laser irradiation of 632.8 and 510 nm wavelengths performed in the 24^{th} hour of germination did not modify the protein content of either the embryo or the endosperm, compared with control seeds. Whereas, the light of 337.1 nm increased the soluble protein content in the embryo, depending on the degree of dose. RNA and DNA contents were not modified by any of these irradiations.

Batov and Kitin (1993) used laser irradiation of rated power density (about 5 mW cm^{-2} and a wavelength lambda = 632.8 nm) to improve the of propagation of false acacia forms (*Robina pseudoacacia* L). An additional irradiation of the upper two or three axillary buds of the cutting not only improved the rooting rate, but also increased the numbers of the adventitious roots and of the root hairs. Secretion of a lesser amount of the rhizogenesis -

timulating phytohormone IAA (indol acetic acid) under laser influence, is upposed.

In 1986-1988 the effect of seed irradiation by laser on the egetative and reproductive manifestations of the plants of the small fruit ultivar and pobeda cucumbers was investigated. Irradiation was performed vith helium - neon laser of 632 nm wavelength and 20 mW power at the utput, and with variants of one, three, five, seven and nine times. The trongest stimulation effect was obtained with five and seven - fold irradiation. Vith these variants the plants formed overground vegetative mass by 10.1 and .5% higher and leaf surface by 25.3 and 28% higher. The higher standard yield fruits are of a length from 3 upto 12 cm) of 20.213 tons/ha (exceeding the ontrol by 16%), was obtained at seven - fold irradiation. The seed irradiation ncreased the content of dry mater, total sugars and vitamin C in the fruits and lastid pigments in their skin (Cholakov, 1990).

The effect of nitrogen laser (337.1 nm) and argon ion laser 514.5 nm) irradiation on physiological response in the green gram *Vigna adiata* L. seedlings was studied by Govil *et al.*, (1991). The shoot and root engths and fresh dry weights of the seedlings increased with 30 min exposure o nitrogen laser and 5 min exposure to argon ion laser. Protein content was naximum with 20 min exposure to N laser and 5 min exposure to argon iron aser, while RNA and DNA contents were maximum at 5 min exposure with oth the laser treatments.

Zubal (1990), reported the results of pot and field experiments elated to studies of the influence of laser irradiation upon winter wheat, spring arley and pea over the years 1986-1988 using the LA 1001 Ne-He laser and

coherent laser beam, at 632.8 nm wavelength, has a biologically stimulant influence resulting in increased emergence velocity as well as in the related dynamics of the beginning of growth. Laser irradiation had no effect upon yields of the studied species and no varietal dependence was found. It is assumed that the described effects of laser irradiation occur in interaction with deteriorated environmental conditions.

Experiments were conducted in 1987-1988 by Nanova (1991, 1992), with alfalfa and a grass mixture (alfalfa, red clover and burr reed) in pots with a capacity of 14 Kg dry soil. Single, repeated and triple seed irradiation with helium - neon laser wavelength 632.8 nm was tested. It was found that pre-seeding laser irradiation of the seeds increased by 85%, the number of alfalfa stems and upto 66% of grass mixture stems as compared to the control. Laser irradiation had no significant effect on plant height and leaf area. As a result of laser treatment accumulation of dry matter increased 27.3% in alfalfa and 16.1% in the grass mixtures. Single laser treatment of the seeds proved more efficient, it increased the root mass of alfalfa (34.5%) and of the grass mixture (17.9%) at the end of the experiments as compared to the control.

2.7 Effect of UV light on plant growth

Radiations of wavelengths below 400 nm wet known to affect plants and animals adversely. Exposure of plants to UV-B had a detrimental effect on growth and biomass accumulation. Under intense UV-B and at 28^0C of temperature, all growth parameters (height, leaf area, fresh and dry weight, stem elongation rate, relative growth rate) of sunflower and maize seedlings

were reduced down to 35% as compared to control. (Mark and Tevini, 1997). Sailemark and Tevini (1997) used *Phaseolus vulgaris* (bush bean) - different cultivars originating from central southern Europe were grown from July to August / September, 1993 upto seven and eight weeks, respectively in two green homes covered by different UV -B absorbing (280 - 320 nm) plastic foils. All the cultivars examined showed significant reduction in height upto 31.87 cms in most growth phases under influence of UV-B. Fresh and dry weights as well as leaf area were also reduced.

Antonelli *et al.*, (1997) conducted an experiment in bean, irradiated with UV radiation after 33 days of treatment, plants grown under UV-B treatment had lower biomass leaf area and reduced leaf elongation compared to UV-A treatment.

2.8 Effect of gamma rays on seed germination plant growth and yield

Gamma rays are a powerful mutagenic agent and attack macromolecules in cells directly. Effects of gamma rays on plant growth and metabolism has also been extensively studied. Like UV radiation gamma rays has detrimental effects on plants.

Borikar and Birajdar, (1983) induced variation in seven polygenic traits by gamma irradiation (10,20 and 30 kR) in cow pea (*Vigna unguiculata)*. The means and variances for plant height, branches and pod number increased with 20 kR dose. The length of the pod was increased with 10 and 20 kR dose.

Induction of physiological damage by γ-rays were confirmed by the observations of Abraham and Desai, (1976); Gaul (1977); Pandey and Gaur, (1984); Sidel (1988) and Geir, (1989). When the seeds of *Abelmoschus*

were treated with γ-rays of lower doses - 10 kR and 20 kR, the seed yield was subsequently increased in M1 generation (Malani *et al.*, 1992). In cowpea the length of pod was increased with gamma ray irradiation (Borikar and Birajdar, 1983). When healthy seeds of french bean were mutagenised with 5-25 kR gamma rays, high protein mutants were isolated in M1 (Prasad and Varma, 1983).

General effects of different doses of γ-radiations with 10, 40 and 50 kR in *Vigna unguculata* was studied and compared with the control R_1 generation (Subramanyam, 1983). The height of the plant, leaf size, pollen size, fruit size and seed size together with yield value decreased in general as the strength of the doses increases. Khamankar (1983) applied 18,25, 32, 39 and 46 kR of γ-rays to the dry seeds of cultivar Kalyansona and Arjun of wheat and their effects on germination, colorptile length and plant height at maturity were studied. All the doses showed significant reduction in germination percentage as compared to control, reduction being progressively more with each succeeding dose. The treatment of 18 kR had no effect on coleoptile length of the plant and maturity remained unaffected.

Dry seeds of Okra variety 2-2 treated with different doses of γ-rays viz. 10,20,30,40,50 and 60 kR indicated that the germination was not affected by radiation treatment, but it was reduced in 60 kR γ-rays treatment. Mortality was increased with higher doses as compared to control. The chlorophyll and fruit mutants were observed with greater frequency in higher doses (Malani *et al.*, 1983).

2.9 Meiotic studies in mutagen treated materials

Bridges/fragments, laggards, abnormal cells and pollen sterility increased with increase in dose of gamma rays, duration of EMS, concentration of sodium azide either alone or in combination was reported by Reddy *et al.*, (1992) and gamma ray treatments exhibited higher irregularities than individual chemical mutagens. Similar results were also obtained in the work of Sharma and Sharma (1979) and Reddy and Annadurai (1992). Sinha and Godward (1969) also noticed a linear relationship between dose/duration/concentration and frequency of aberration. The abnormalities were high at higher level of mutagens and the increase was more in combined treatments as compared to individual treatments. Earlier observations in lentil by Sinha, (1967) and Acharia and Sinha, (1975) and various other crops such as corn by Beadle, (1930) and rye by Prakken, (1943) also support the work of Reddy *et al.*, (1992). Jana *et al.*, (1974) observed a linear increase in chromosomal abnormalities with exposure to gamma rays in lentil.

The increase in pollen sterility could be attributed to increase in cytological abnormalities (Reddy *et al.*, 1992). The degree of chromosome paring was shown to be mainly responsible for increased sterility in mutagenic population of black gram (Jana, 1963). Cherry and Bhalla (1988) also noticed increase in pollen sterility with increase in mutagenic level. Gaul *et al.*, (1966) and Ekberg (1969) suggested that sterility following mutagenic treatments might be attributed to detectable chromosomal aberration and cryptic deficiencies.

MATERIALS AND METHODS

3.1 Materials

Taxonomically diverse plant species suitable for cytological analysis, namely *Vicia faba* (faba bean) belonging to the family Leguminosae, sub family Fabaceae (family Leguminosae) and *A. cepa* L. syn. *aggregatum* (onion or chives) belonging to Liliaceae family, were selected as the experimental material.

Seeds of *V. faba* var. VH 82-1 were obtained from the Medicinal and Aromatic Plant Section, Department of Plant breeding, Haryana Agricultural University, Hisar, India and bulbs of *A. cepa* L. syn. *aggregatum* var. CO_4 were obtained from the College of Horticulture, Tamilnadu Agricultural University, Coimbatore, India. The *V. faba* seeds were stored at low temperature (4°C) and *A. cepa* bulbs were stored in cotton bags at room temperature till use.

3.2 Germination of seeds and sprouting of bulbs

Seeds of *V. faba* wrapped in cotton cloth were soaked in water for 12 hours. The soaked seeds were then decoated and placed in petriplates on moist filter paper. The petriplates were incubated at 25±2°C for germination. From mature bulbs, the outer loose dry scale leaves and old roots were removed to facilitate rooting. For emergence of sprouts, the bulbs were placed in moist soil.

3.3 Mutagens used

Five mutagens - three physical mutagens - γ-radiation, Laser radiation and UV radiation and two chemical mutagens - Ethyl Methane Sulponate and Hydroxyl amine were used for the present study.

3.4 Irradiation of samples by physical mutagens

3.4.1 γ-irradiation

Gamma irradiation was done at the Bhabha Atomic Research, Centre, Mumbai, India. Dry seeds of *V. faba* and bulbs of *A. cepa* were irradiated at doses 2,5,10,15 and 20kR. Treated seeds were stored in fridge at 4^0C and bulbs in a cotton bag at room temperature till use.

3.4.2 Laser irradiation

Laser irradiation was done at the International School of Photonics, Cochin University of Science and Technology, Cochin, India. Argon ion laser source (Spectra Physics Model - 171) was used for the irradiation *V. faba* seeds were soaked over night in tap water and decoated before irradiation. Old roots and outermost old scale leaves were manually removed in the case of *A. cepa* bulbs and were planted in moist sand over night to encourage root and shoot initiation before irradiation.

The embryo portion of the seeds and the apical bud portion of the bulbs were exposed to 476, 488 and 514 nm excitations from Argon ion laser source. In 514 nm various power levels - 200, 400, 600 and 800 mW with different power densities 2.25, 4.49, 6.74 and 8.89 mW cm^{-2} and different exposure times - 10, 20, 30 and 40 minutes exposures were tested. In 476 and 488 nm excitations the power levels 200 and 400 mW with the corresponding power densities and exposure times of 10, 20, 30 and 40 min were tested. The Laser beam size was adjusted using proper optical elements so as to get uniform illumination in the sample container.

3.4.3 UV irradiation

The UV irradiation was done on materials subjected to pre-germination process done as in the case of laser irradiation. The embryo and apical bud portion of the materials were exposed to 30 Watt UV lamp attached to laminar air flow for 1,2 and 3 hours at a distance of 5 cm from the source.

3.5 Chemical mutagens treatment of materials

The samples for chemical treatments were also subjected to pre-germination/sprouting processes as in the case of the aforesaid methods. The samples were then soaked in 0.2, 0.4, 0.6, 0.8 and 1% of Ethyl Methane Sulphonate solutions for 30 minutes and the same concentrations of Hydroxyl amine solutions for 1 hour. The solutions were prepared in distilled water.

3.6 Sprouting/germination of treated material

Immediately after the various treatments, the onion bulbs were replanted in moist sand and the faba bean germinated on moist filter paper in petridishes at $25\pm2^{0}C$. In the case of γ-irradiated samples however, the materials were kept for germination only just prior to collection of root tips for analysis.

3.7 Collection of root tips for mitotic studies

Onion root tips of length 1-2 cm were collected between 8 and 9 A.M on the 3rd day while in faba bean root tips of the same size were collected between 10 and 11 A.M after 3-5 days of treatments.

3.7.1 Pre-treatment of root tips

The root tips thus collected were pre-treated in 0.04% colchicine solution for about 3 hours at room temperature.

3.7.2 Fixation of pre-treated root tips

The root tips were washed twice with distilled water for removing the pre-treating agent and were fixed in a mixture of 3 parts of absolute alcohol and 1 part of glacial acetic acid. Fixative was prepared fresh every time. Root tips could be kept in the fixative upto 15 days in the refrigerator. Some of the root tips were fixed directly without colchicine pre-treatment for anaphase study.

3.7.3 Preparation of root tip squashes

From the fixative, the roots were transferred to distilled water and were washed twice. They were then hydrolysed in 1N Hydrochloric acid at about 60^0C for a few seconds. After hydrolysis, the root tips were washed twice in distilled water and then transferred to distilled water.

On a clean slide, the tips were separated from the rest of the root and crushed in a drop of 2% acetocarmine with the flat end of a rod and squashed under a coverslip. The pressure was applied under several thickness of blotting paper. Sideways movement of coverslip was avoided in order to prevent the rolling of cells.

3.8 Photomicrography

Photographs were taken from temporary slides. Camera of the model Nikon F-601M was used for taking photographs Film ORWO NP 22 and

NOVA FP4 with the speed of ASA (ISO) 125 DIN 22 was used. All the photographs were taken under oil immersion lens.

3.9 Scoring of slides

For scoring of cytological aberration, temporary slides were used. Atleast 6 slides were prepared from actively dividing root tips in each dose and 15-20 fields (approximately 5,000-10,000 cells) were scored. Different structural changes of chromosomes were scored at metaphase and anaphase. Micronuclei were scored at anaphase/telophase. Savage's (1975) classification of various types of chromosomal aberration was used for scoring the aberration. Mitotic index (M.I.) was calculated as below:

$$M.I. = \frac{\text{Number of dividing cells}}{\text{Total no. of cells}} \times 100$$

3.10 Meiotic studies

Selected treatments of the mutagens were used for the meiotic studies. The treatments included : Laser irradiation at two doses (viz. 10 min exposures at power density 200 mW and 30 min exposure at power density 400 mW) in each wavelength of 476 nm, 488 nm and 514 nm., UV exposures of 1 hour and 3 hour, γ-irradiation at 2,5, 10, 15 and 20 kR, EMS treatment at doses 0.2% and 1% and Hydroxyl amine treatment at doses 0.2% and 1%.

Young flower buds were collected between 8 & 10 A.M. and the flower buds were fixed in absolute ethyl alcohol : acetic acid (3:1) mixture for at least 24 hours. The fixed flower buds were stored in fridge at ~ 4°C for subsequent use. Anthers were squashed in 1% iron-acetocermine. Temporary slides were used for scoring. Several slides were prepared in each treatment.

Photomicrographs were taken from temporary slides as in the case of mitotic studies.

3.11 Pollen stainability

Pollen stainability was determined using 1% iron acetocarmine smear technique. Atleast 3 smears were prepared in each dose and many fields in each smear were counted. The mean of these values were used for determining the percentage of sterility of pollen grains.

3.12 Germination/Sprouting index, Growth and Yield studies

Germination/sprouting index growth and yield was monitored in selected treatments of the mutagens used in this study. The treatments included : laser irradiation at two doses (viz. 10 min exposure at power density 200 mW and 30 min exposure at power density 400 mW) in each wavelength of 476 nm, 488 nm and 514 nm, UV exposure of 1 hour and 3 hour, γ-irradiations at 2,5, 10, 15 and 20 kR, EMS treatment at doses 0.2% and 1% and Hydroxyl amine treatment at doses 0.2% and 1%.

25 seeds of *V. faba* and 20 bulbs of *A. cepa* in each treatment were sown in the field at the Indian Agricultural Reserch Institute Regional Station, Wellington, The Nilgiris, Tamilnadu, India at a spacing of 20 cm between plants and 50 cm between rows.

The germination/sprouting index was computed using the equation:

$$\frac{\text{No of seeds(bulbs) showing seedling(sprouting)}}{\text{Total No. of seeds(bulbs)}}$$

Growth was monitored as the change in plant height during the crop duration.

Yield was calculated on the basis of the equation:

$$\frac{\text{Total weight of seeds(bulbs) in each treatment}}{\text{Total number of plants in each treatment.}}$$

Two repeats of each treatment were done.

3.13 Estimation of soluble protein content and assay for enzyme activity

The estimation of soluble protein content and assays for enzyme activity of protease, amylase, peroxidase and catalase were done in both plant species in selected mutagen treatments. The treatments included: laser irradiation at two doses (Viz. 10 min exposure at power density 200 mW and 30 min exposure at power density 400 mW) in each wavelength of 476 nm, 488 nm and 514 nm, γ-irradiation at 2,5, 10, 15 and 20 kR and EMS treatment at doses 0.2% and 1%.

The estimation of protein content and activity of various enzymes were assayed separately in embryo and cotyledon in *V. faba* while in *A. cepa* it was assayed separately in the inner true leaves with apical bud and in outer fleshy scale leaves. Amylase assay was done on second day and fourth day of germination, protease on third day and sixth day and peroxidase and catalase on fifth day of germination in *V. faba* while in *A. cepa* estimation of protein content and assay of these enzymes were done on second day and fourth day of sprouting. Peroxidase activity in *A. cepa* bulbs treated with EMS was done only on fourth day of sprouting.

3.13.1 Enzyme extraction

Germinated seeds of *V. faba* and sprouted bulbs of *A. cepa* were harvested on specified days. The seeds were dissected into embryo and cotyledon and bulbs into inner true leaves with apical bud and outer fleshy scale leaves on aluminium foil placed on ice. The two samples were then cut into pieces with the help of scissors, weighed and homogenised using suitable buffer in a precooled mortar and pestle. The slurry thus obtained was centrifuged at 10,000 rpm for 30 min at 4^0C and the supernatant used as enzyme source.

3.13.2 Protein estimation

Protein content was estimated by the method of Lowry et al, (1951). 0.2 ml of test sample was made up of 1.0 ml with distilled water to which 2 ml protein reagent (prepared by mixing 0.1 N sodium hydroxide containing 2% sodium carbonate, 1% copper sulphate and 2% potassium sodium tartate in the ratio 100:1:1) was added. After 10 min incubation, 0.5 ml Folin's reagent (1:2 dilution) was added and the samples incubated for 30 min at room temperature. The blue colour developed by the reduction of phosphomolydbic - phosphotungstic component in the Folin - Ciocalteau reagent by the aminoacids tyrosine and tyrtophan present in protein was measured in a spectrophotometer at 660 nm. Concentration of protein was determined from a standard graph prepared using Bovine Serum Albumin.

3.13.3 Assay for Protease activity

Protease activity was assayed by a modification of the method of Dubey (1982). Casein according to Hammersten is used as substrate for protease. 1% casein solution was prepared in 0.1 M phosphate buffer (pH 7.6)

by heating for about 15 min in a boiling water bath until casein was dissolved. It was then diluted to 100 ml. Enzyme solution was prepared by extracting 150 mg sample in 1 ml 0.1 M phosphate buffer (pH 7). 0.1 ml substrate solution and 0.2 ml enzyme solutions were pipetted into test tubes. Control contained only substrate solution. The reaction mixture was incubated for one hour in a water bath at 37^0C. Then 0.6 ml 5% TCA was added to arrest reaction and kept for half an hour. The mixture was centrifuged at 10,000 rpm for 10 min The TCA soluble peptide fragments are measured at 660 nm by the method of Lowry *et al.,* (1951). One unit of protease is expressed as micrograms of peptide fragments formed in 60 min incubation time and specific activity is expressed as activity/mg protein.

3.13.4 Assay for Amylase activity

Amylase assay was done by a method described by Sadasivam and Manickam (1992). 200 mg sample was extracted in 1.0 ml 66 mM phosphate buffer (pH 7) containing 0.5 M NaCl. The pellet was extracted with phosphate buffer containing 0.5% mercaptoethanol. The supernatants of two extractions were collected and mixed. To 1.0 ml 1% starch solution (prepared in acetate buffer, pH 4.7) 0.1 ml enzyme solution, diluted to 1.0 ml with phosphate buffer was added. The mixture was incubated at 27^0C for 15 min The reaction was stopped by addition of 2 ml dinitrosalicylic acid reagent. The solution was heated in boiling water bath for 5 min after which 1 ml 40% sodium potassium tartrate was added. After cooling the tubes, 6 ml of distilled water was added.

Absorbancy was read in a spectrophotometer at 560 nm. Concentrations were measured from the standard graph prepared with 0-

1000 μg maltose. One unit of amylase is expressed as μg of maltose produced during 15 min incubation with 1% starch and specific activity as activity per mg protein.

3.13.5 Assay for Peroxidase activity

Assay for peroxidase was done by a method described by Sadasivam and Manickam (1992). Guaiacol was used as substrate for the assay.

Guaiacol + H_2O_2 → oxidised guaiacol + $2H_2O$

The rate of formation of guaiacol dehydrogenation product is a measure of peroxidase activity and can be assayed spectrophotometrically at 436 nm.

1g of fresh tissue was ground in 0.5 ml of 0.1 M phosphate buffer (pH 7) and the slurry centrifuged at 10,000 rpm for 30 min at 4^0C. The supernatant was used as enzyme source. 3 ml of 0.1 M phosphate buffer, 0.05 ml of 20 mM guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml of 12.3 mM H_2O_2 solution was pipetted into a cuvette (H_2O_2 solution was freshly prepared by diluting 0.14 ml 30% H_2O_2 to 100 ml with distilled water), mixed and placed in a spectrophotometer, was adjusted to wavelength 436 nm. A stop watch was started and the time required to increase optical density by 0.1 is noted.

Since the extinction coefficient of guaiacol dehydrogenation product at 436 nm under conditions specified is 6.36 per micromole, the enzyme activity per litre of extract was calculated as below.

$$\text{Enzyme activity (units/litre)} = \frac{3.18 \times 0.1 \times 100}{6.39 \times 1 \times \Delta t \times .1} = \frac{500}{\Delta t}$$

.6 Assay for Catalase activity

Catalase assay was done by permanganate titration method. Catalase decomposes hydrogen peroxide to water and oxygen.

1g of tissue was extracted in 0.5 ml of 0.1 M phosphate buffer (pH 7). 15 ml of 0.01 N hydrogen peroxide was placed in an Erlenmeyer flask. 0.1 ml of catalase solution was added to it, while simultaneously starting a stop watch. After two min, 2 ml of the solution was withdrawn and added to an excess of 2 % sulphuric acid for titration with 0.01 N potassium permanganate. The quantity x_1 is determined. Then 2 ml of the solution was again withdrawn after 4 min and titrated as above to determine the quantity x_2.

The kinetics of hydrogen peroxide disappearance closely follow first order kinetics and the velocity constant (K) is calculated.

$$K = \frac{2.3}{t_2 - t_1} \log \frac{x_1}{x_2}$$

The units are sec^{-1}. One unit is taken as the amount of enzyme which liberates half the peroxide oxygen from a hydrogen peroxide solution of any concentration in 100 sec at 25^0C. The unit of enzyme activity is therefore related to the half life (τ) of the first order reaction for τ the following formula is valid.

$$\tau = \frac{\ln 2}{K} = \frac{0.693}{K}$$

The relationship between observed half life time τ observed and enzyme activity is,

$$1 \text{ unit} = \frac{100}{\tau \text{ observed}} = \frac{K \text{ observed}}{6.93 \times 10^{-3}}$$

RESULTS

4.1 Mitotic chromosome complement of the materials

Mitotic chromosome complement of *A. cepa* contains 16 chromosomes which could be classified into seven pairs of submetacentric chromosomes and one pair of telocentric chromosomes with a secondary (nuclear) constriction that separates a satellite from the rest of the chromosomes (plate. fig. 1).

The mitotic chromosome complement of *V. faba* contains 12 chromosomes which could be classified into five pairs of nearly equally long chromosomes with subterminal centromeres (chromosomes II - VI, also referred to as the S chromosomes) and one pair with median contromere (chromosomes I, also called the M chromosome). The M chromosome contains a secondary (nucleolar) constriction that separates a large satellite from the rest of the chromosome (plate. fig. 43).

4.2 Mitotic chromosomal aberration

In control (untreated) samples of *A. cepa* and *V. faba,* mitosis was normal with only 0.17 and 0.11% of aberration observed respectively.

In treated samples, a wide range of chromosomal aberration were observed in all the four stages of mitotic cell division. The most important changes observed were : clumping, stickiness and nondisjuction of chromosomes, bridges, laggards, micronucleate, binucleate and elongated nucleate cells. In addition to these some other types of aberration such as interphase with unequal sized nuclei, polyploid cells, chromatin droplets,

nuclear bridges, nuclear polymorphism and multinulceate conditions were also encountered in low frequencies.

4.2.1 Prophase aberration

4.2.1.1 Clumping of chromosomes

The most important type of chromosomal aberration observed at prophase was clumping of chromosomes (plate. figs.8, 48, 66, 81, 89). The frequency of this change was very low (0.03%) in control (untreated) samples of both plant species.

In *A. cepa,* laser exposures at 476 nm induced an increase in clumping of chromosomes with increase in power density and exposure time (Fig.1A App.IA). However lasers at 488 nm did not show dose dependence (Fig.2A App.IIA) and at 514 nm there was a dose dependent decrease in clumping of chromosomes with increase in power density (Fig.3A App.IIIA). The frequency of chromosome clumping was similar at 476 and 488 nm and was several fold lower than at 514 nm. In *V. faba,* this parameter showed a dose dependent increase with laser irradiation at 476 nm (Fig.1B App.IB) although no dose dependence was seen with lasers of 488 nm (Fig.2B App.IIB). On irradiation at 514 nm, a dose dependent increase was noticed at 200 mW although higher power densities caused a decrease in chromosome clumping with increase in exposure time (Fig.3B App.IIIB). Clumping frequencies were lowest in 476 nm and highest in 514 nm.

Exposure to UV for different durations in *A. cepa* elicited a sharp increase in chromosome clumping at 2 hours (Fig.4A App.IVA). The frequency of chromosomes clumping at 1 and 3 hours of UV irradiation was similar to that

induced by lasers of 476 and 488 nm. The frequency at 2 hours of UV exposure was similar to that induced by lasers of 514 nm. In *V. faba,* clumping frequency showed dose dependent decrease with increase in time of exposure to UV rays. The frequency was similar to that caused by lasers of 476 nm and was 2-3 times lower than that induced by laser exposure at 488 and 514 nm.

γ-irradiation of *V. faba* induced dose dependent increase in the frequencies of chromosome clumping (Fig.5B App.VB). The clumping frequency elicited by 476 nm laser exposure was similar to the frequency induced by different doses of γ-irradiations. Laser exposures of 488 and 514 nm caused chromosome clumping to a higher frequency than γ-irradiations.

EMS treatments in *A. cepa* induced an increase in chromosome clumping with increase in concentration (Fig.5A App.VA). Clumping frequencies induced by laser exposures of 476 and 488 nm were lower and 514 nm higher than that induced by EMS treatments. In *V. faba* also, EMS treatments induced an increase in clumping frequency with increase in concentration (Fig.6B App.VIB). Clumping frequencies induced by lasers exposures at 476 nm and EMS treatments were similar, while that of 488 and 514 nm were higher than the frequencies induced by EMS treatments.

HA treatments induced an increase in clumping frequency with increase in concentration in *A. cepa* (Fig.6A App.VIA). The frequency of chromosome clumping caused by HA was similar to that induced by lasers of 476 and 488 nm but lower than that induced by 514 nm. In *V.faba* also, HA treatments induced an increase in clumping frequency (Fig.7B App.VIIB). The clumping frequency caused by laser exposures at 476 nm was similar to and

Fig. 1 A : Effect of laser irradiation at 476 nm on clumping of chromosomes in *A. cepa*

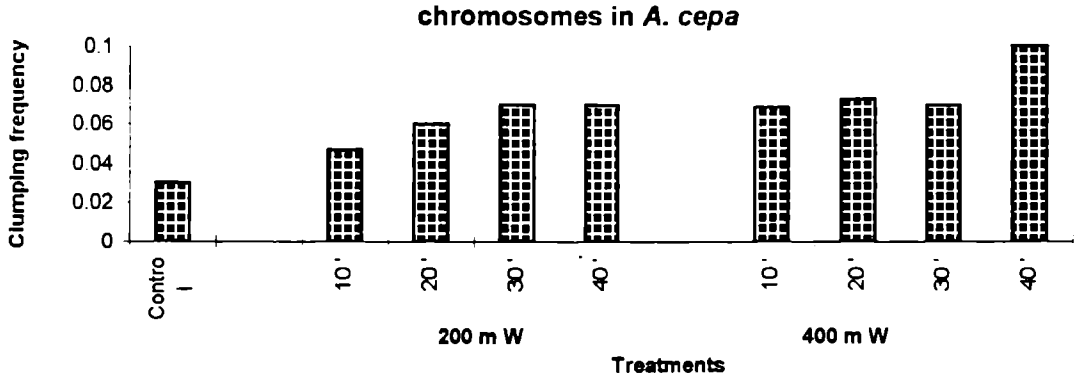


Fig. 2 A : Effect of laser irradiation at 488 nm on clumping of chromosomes in A. cepa

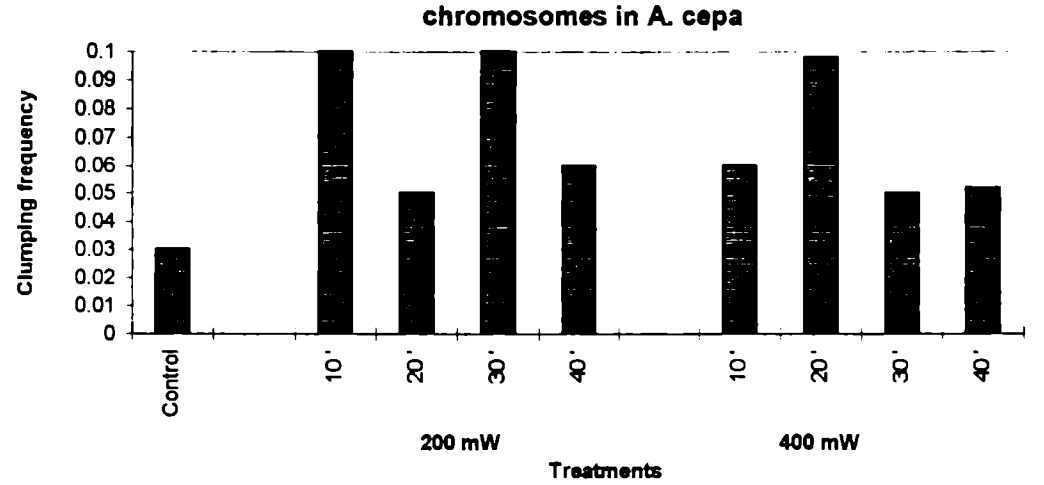


Fig. 3 A : Effect of laser irradiation at 514 nm on clumping of chromosomes in *A. cepa*

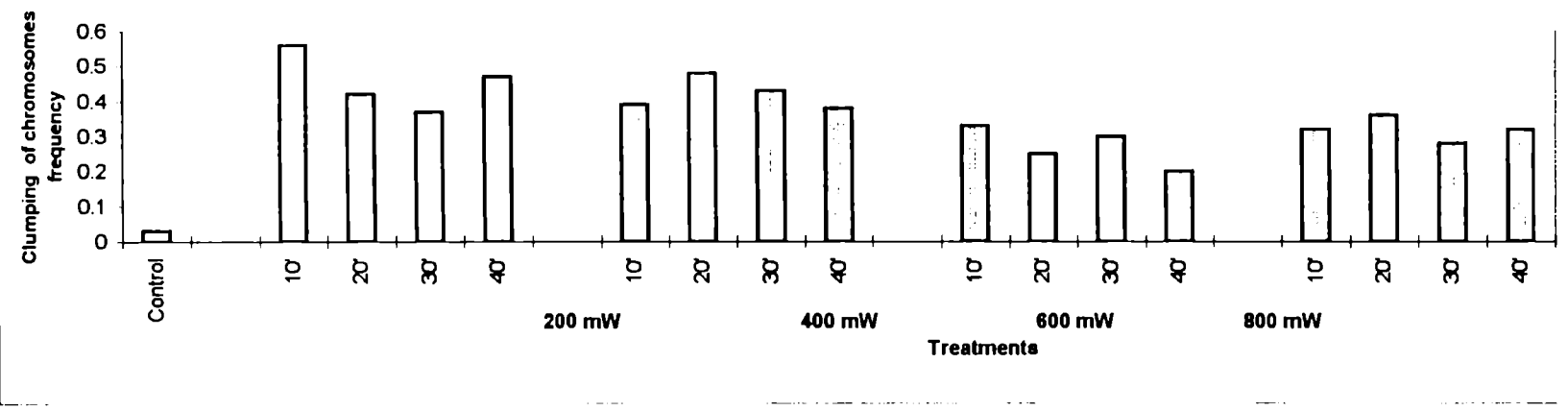


Fig.4 A : Effect of UV exposures on clumping of chromosomes in *A. cepa*

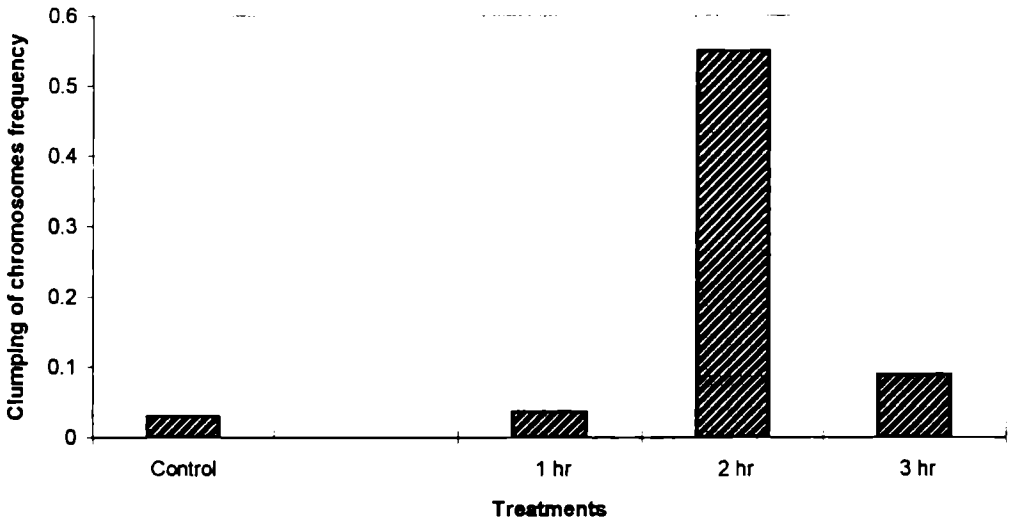


Fig.5 A : Effect of EMS treatment on clumping of chromosomes in *A. cepa*

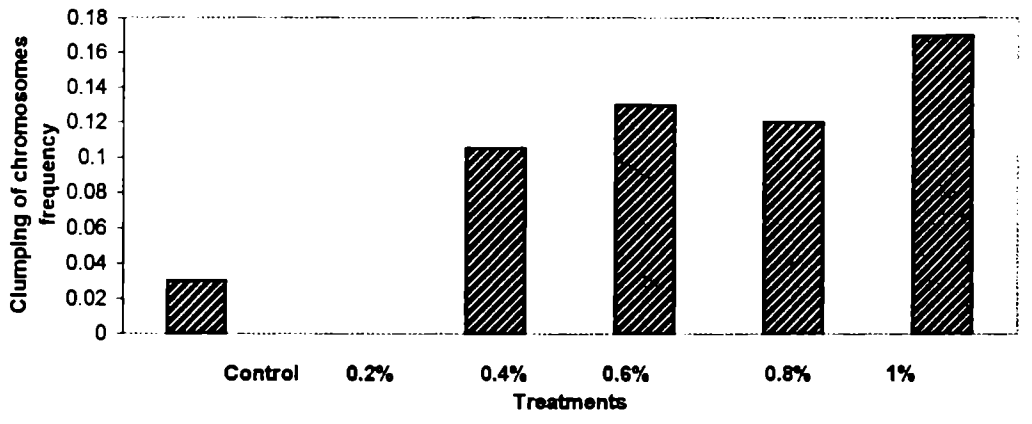
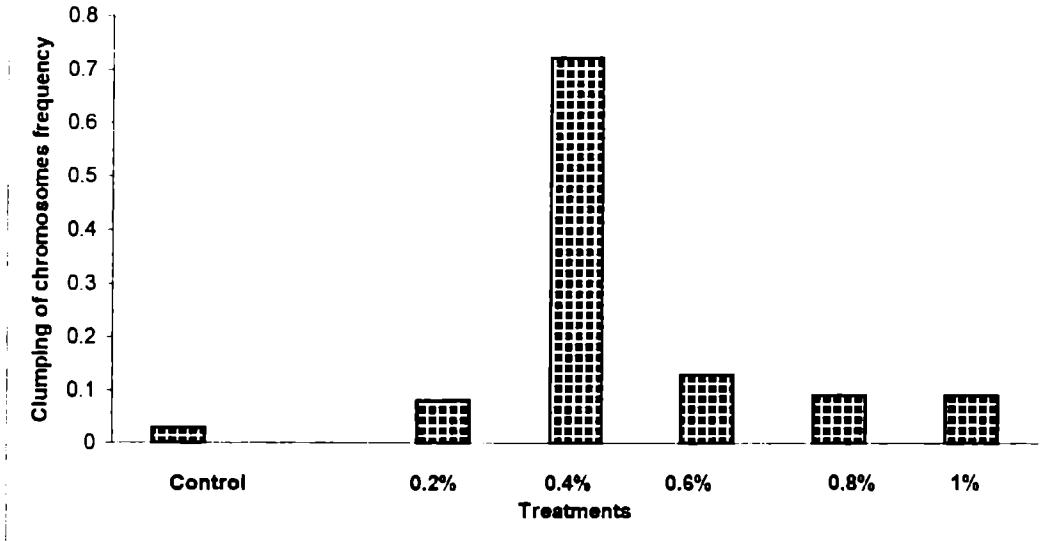


Fig. 6A : Effect of HA treatments on clumping of chromosomes in *A. cepa*



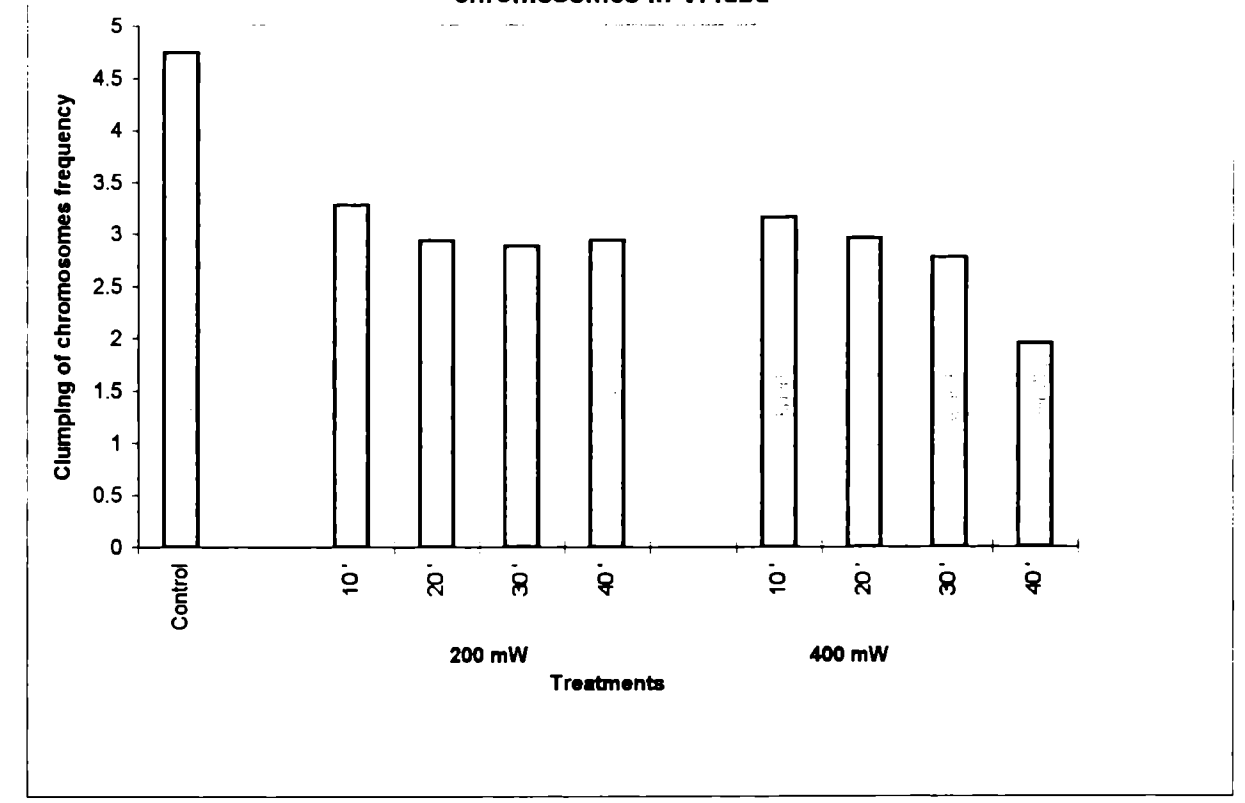


Fig. 1 B : Effect of laser exposure at 476 nm on clumping of chromosomes in V. faba
Clumping of chromosomes frequency
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Control
10'
20'
30'
40'
10'
20'
30'
40'
200 mW
400 mW
Treatments

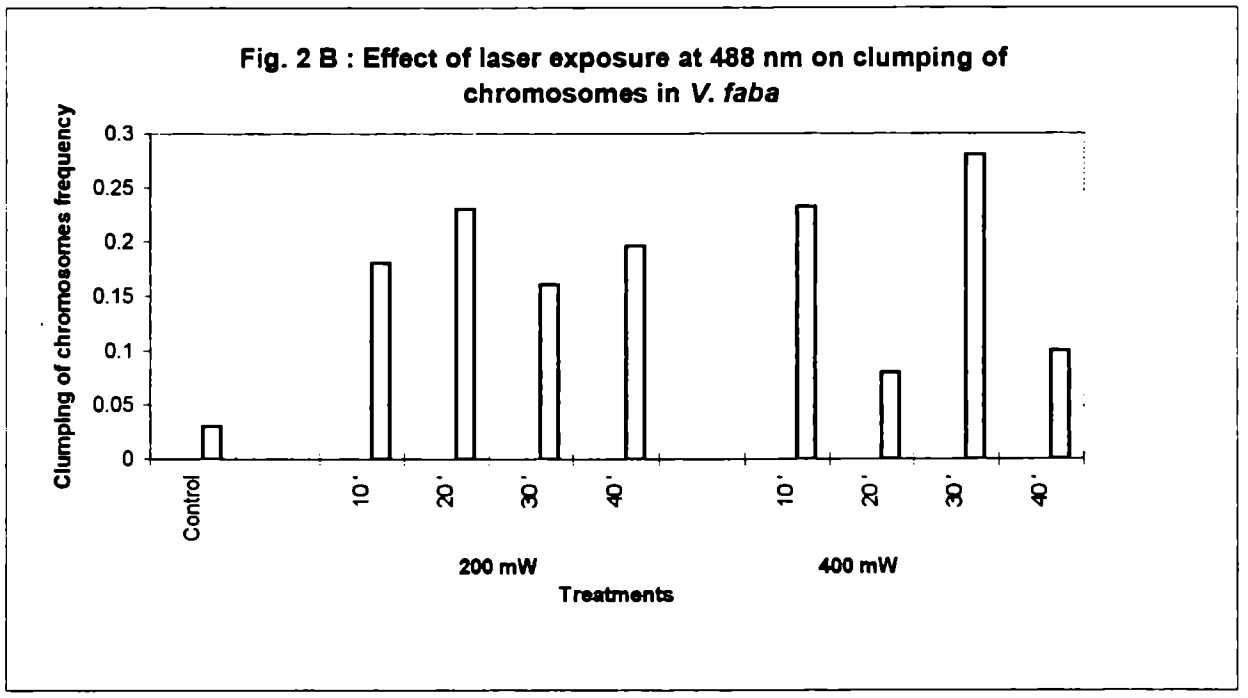


Fig. 2 B : Effect of laser exposure at 488 nm on clumping of chromosomes in V. faba
Clumping of chromosomes frequency
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
10'
20'
30'
40'
10'
20'
30'
40'
200 mW
400 mW
Treatments

Fig. 3 B : Effect of laser irradiation at 514 nm on clumping of chromosomes in *V. faba*

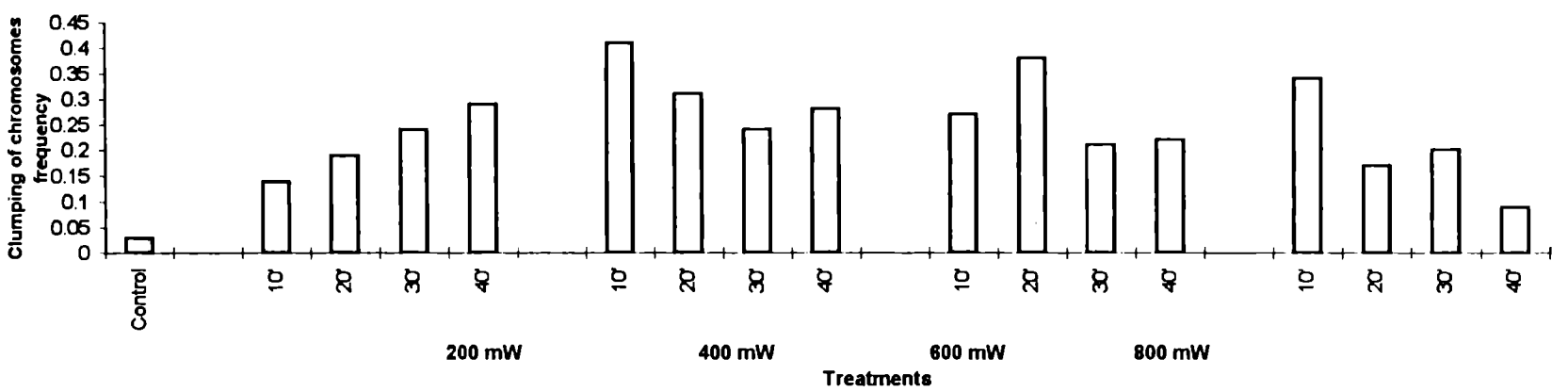


Fig. 4 B : Effect of UV exposure on clumping of chromosomes in *V. faba*

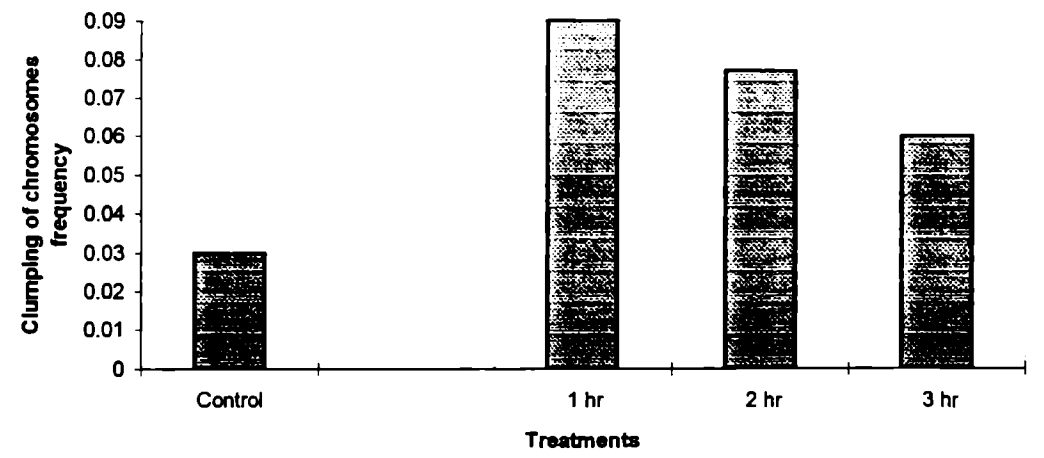


Fig. 5 B : Effect of gamma irradiation on clumping of chromosomes in *V. faba*

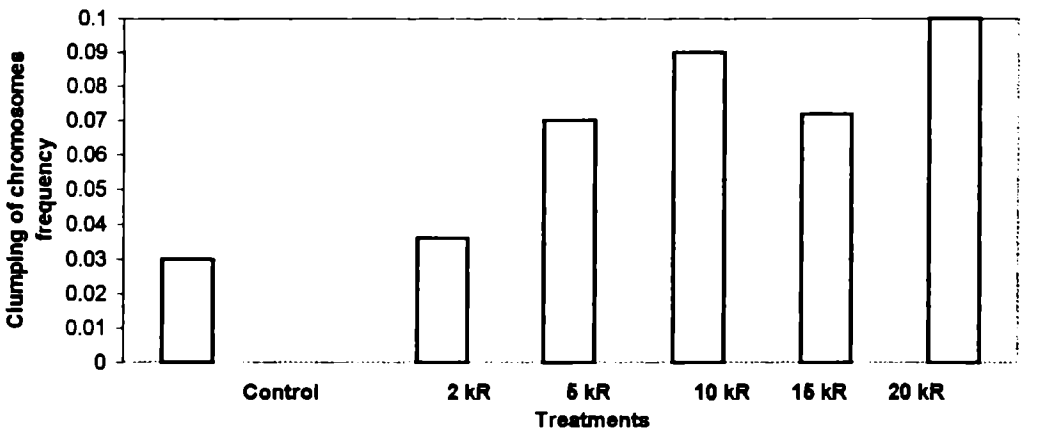


Fig. 6 B : Effect of EMS treatment on clumping of chromosomes in *V. faba*

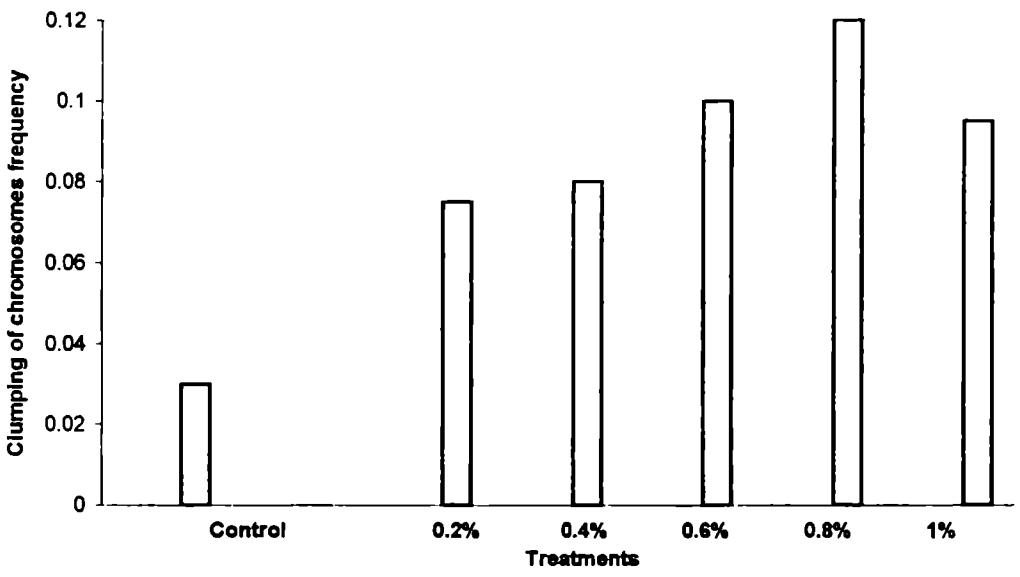
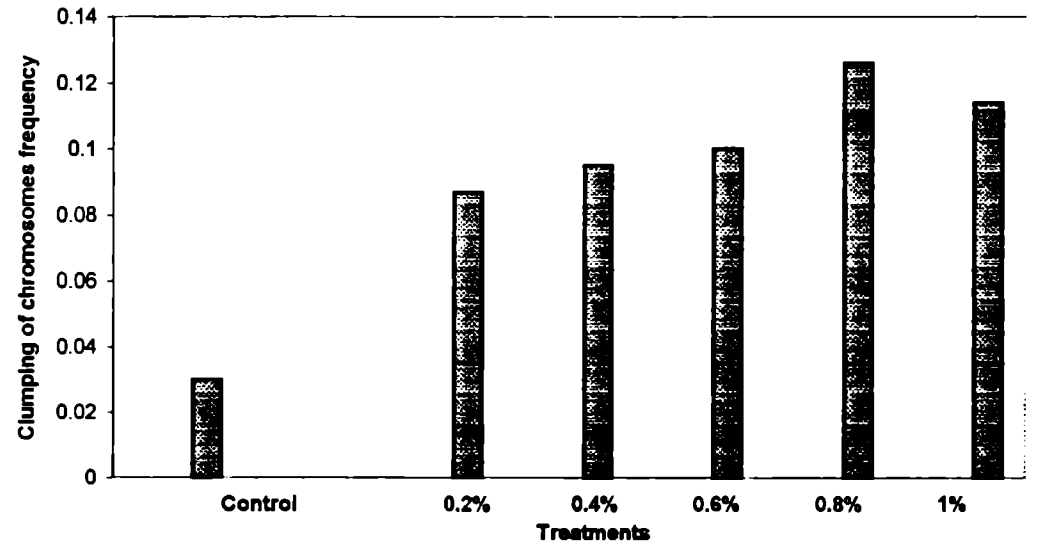


Fig. 7 B : Effect of HA exposure on clumping of chromosomes in *V. faba*



that induced by 488 and 514 nm were higher than the frequencies induced by HA treatments.

By comparing the clumping frequencies in *A. cepa* and *V.faba*, laser exposures at 476 nm caused a dose dependent increase, while no dose dependence could be established in both plant species in the clumping frequencies induced by 488 nm lasers. A dose dependent decrease in *A. cepa* was observed for laser irradiation at 514 nm but not in *V. faba*. UV exposures did not cause a dose dependent change in chromosome clumping in *A. cepa* but in *V. faba* a dependent decrease was observed. EMS and HA treatments caused dose dependent increase in chromosome clumping in both plant species.

4.2.2 Metaphase aberration

4.2.2.1 Stickiness of chromosomes

The most common type of chromosomal aberration observed at metaphase was stickiness of chromosomes (plate.figs. 3, 9, 10, 11, 21, 30, 38, 44, 54, 55, 68, 69, 70, 71, 83, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 103). It was totally absent in the control but was encountered in all the mutagen treatments.

In *A. cepa* laser exposure at 476 nm induced an increase in stickiness of chromosomes with increase in power density (Fig.7A App.IA). Laser exposure at 488 nm induced a dose dependent decrease in stickiness of chromosomes with increase in power density (Fig.8A App.IIA) and that of 514 nm laser exposure induced a dose dependent increase of the same parameter at 200 and 400 mW and no dose dependent change at 600 and 800 mW (Fig. 9A App.IIIA). The frequency of this change induced by lasers at 488 nm

was higher than that induced by 476 and 514 nm. Also, chromosome stickiness frequency caused by 476 nm laser exposure was higher than that induced by 514 nm.

In *V. faba* laser exposure at 476 nm induced an increase in stickiness with increase in power density (Fig.8B App.IB), while at 488 nm of laser exposure there was a dose dependent decrease in stickiness with increase in power density (Fig.9B App.IIB). On irradiation at 514 nm, stickiness decreased with increase in exposure time (Fig.10B App.IIIB). Stickiness frequencies were highest at 488 nm and lowest at 514 nm.

In *A. cepa* stickiness frequency showed dose dependent decrease with increase in exposure time of UV rays (Fig. App.). The frequency of this change was lower than that caused by lasers of 476, 488 and low power densities of 514 nm and similar to high power densities of 514 nm. In *V. faba* stickiness showed dose dependent decrease with increase in time of exposure to UV rays (Fig. IIB App. IV B). The frequency elicited by UV was lower than the frequency of this change caused by laser exposures.

γ-irradiations in *V. faba* samples produced dose dependent increase in the frequency of stickiness (Fig.12B App.VB). The frequency was higher than that of the frequencies of this change caused by laser exposures.

In *A. cepa*, stickiness frequency showed dose dependent increasd with increase in EMS concentration (Fig.IIA App.VA). The frequency was higher than that caused by lasers of 476 and 514 nm and similar to 488 nm. In *V. faba* also, EMS treatments induced dose dependent increase in stickiness frequency

Fig. 7A : Effect of laser irradiation at 476 nm on stickiness of chromosomes in *A. cepa*

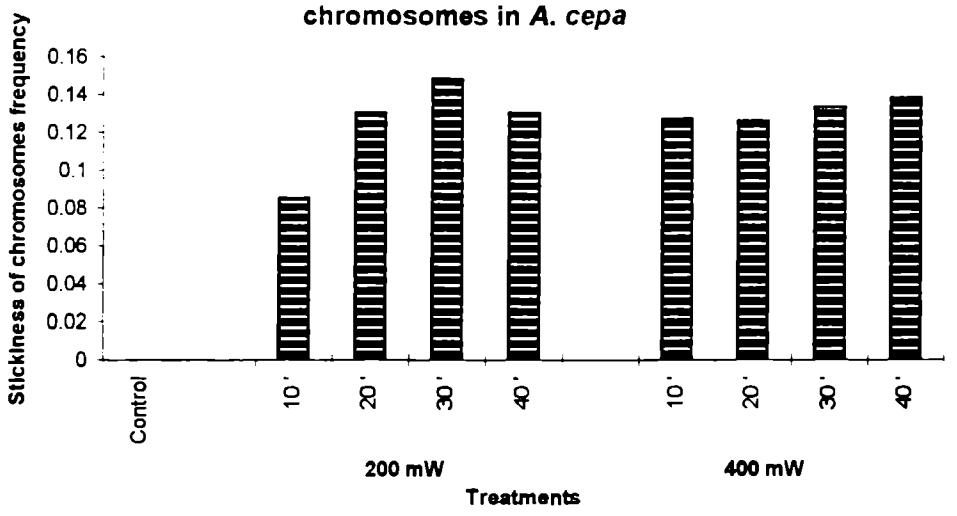
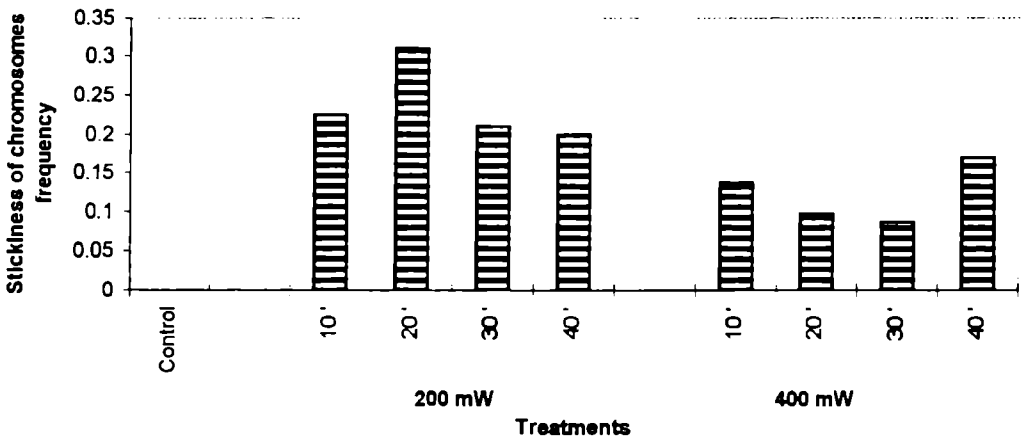


Fig. 8A : Effect of laser irradiation at 488 nm on stickiness of chromosomes in *A. cepa*



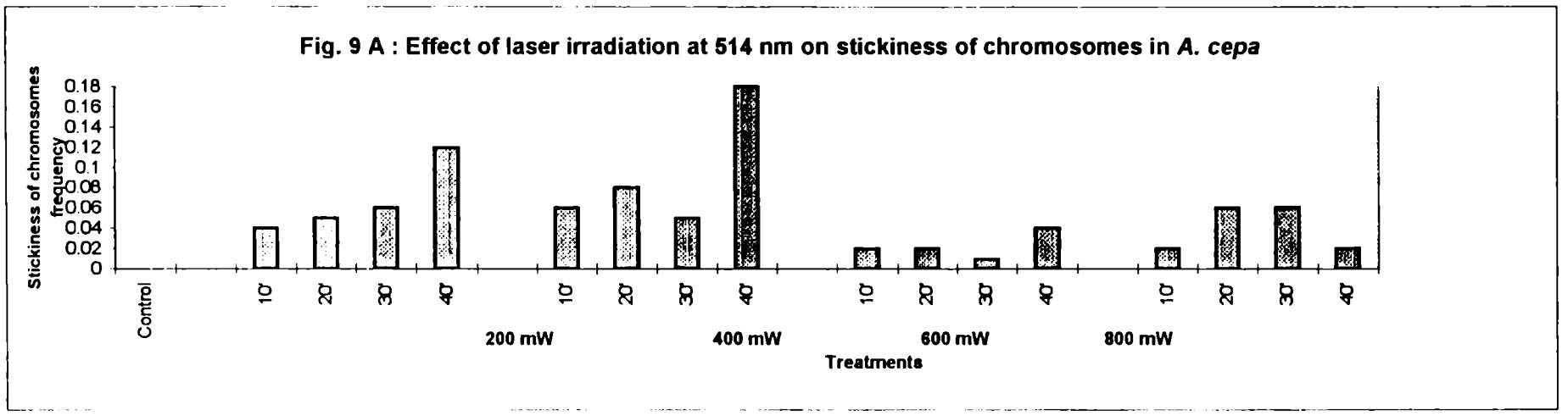


Fig. 9 A : Effect of laser irradiation at 514 nm on stickiness of chromosomes in A. cepa
Stickiness of chromosomes frequency
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Control
10'
20'
30'
40'
200 mW
400 mW
600 mW
800 mW
Treatments

Fig. 10 A : Effect of UV exposures on stickiness of chromosomes in *A. cepa*

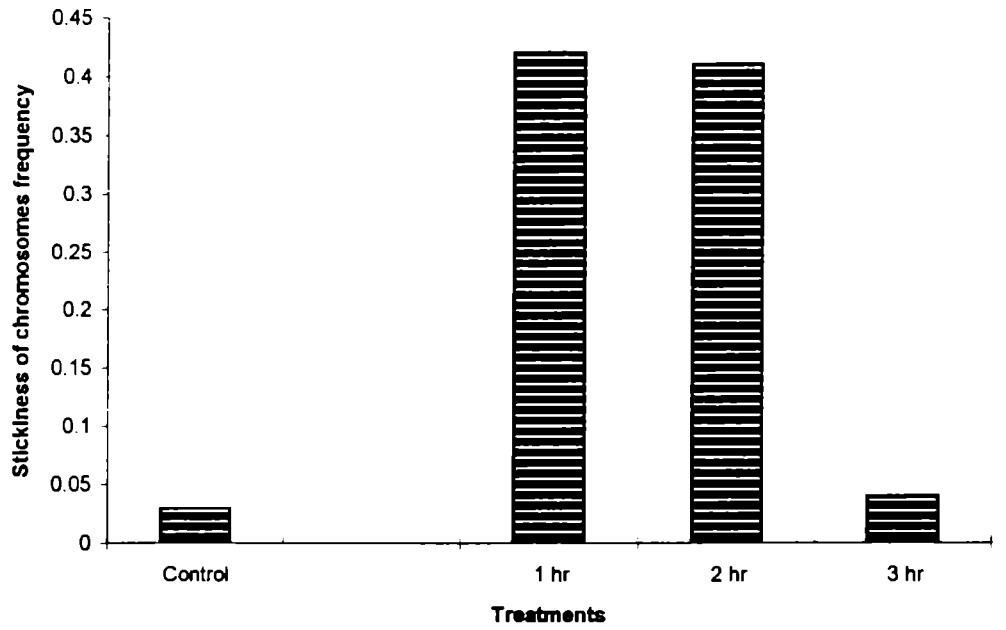


Fig. 11 A : Effect of EMS treatments on stickiness of chromosomes in *A. cepa*

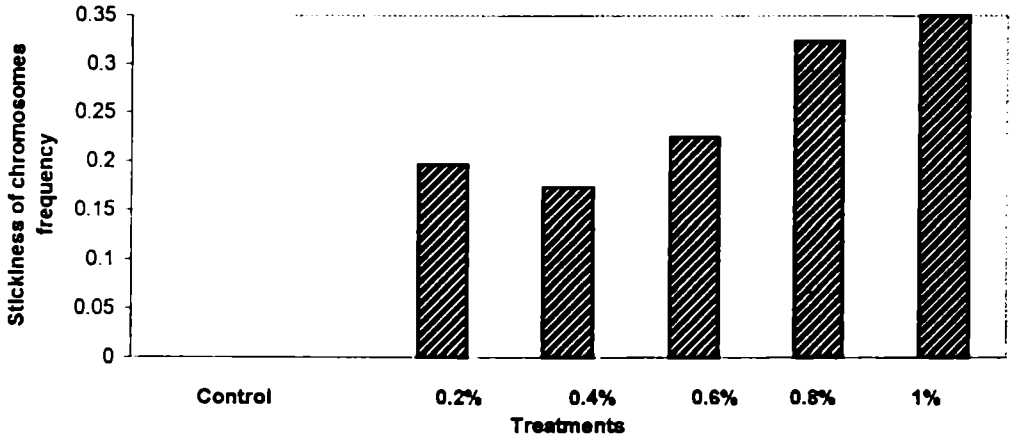


Fig. 12 A : Effect of HA on stickiness of chromosomes in *A. cepa*

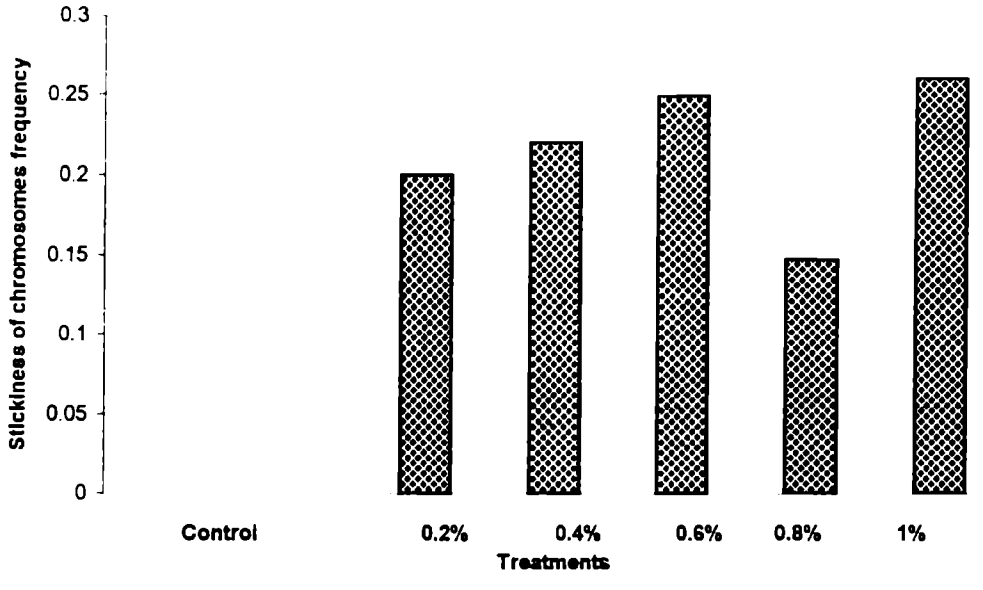


Fig. 8 B : Effect of laser exposure at 476 nm on stickiness of chromosomes in *V. faba*

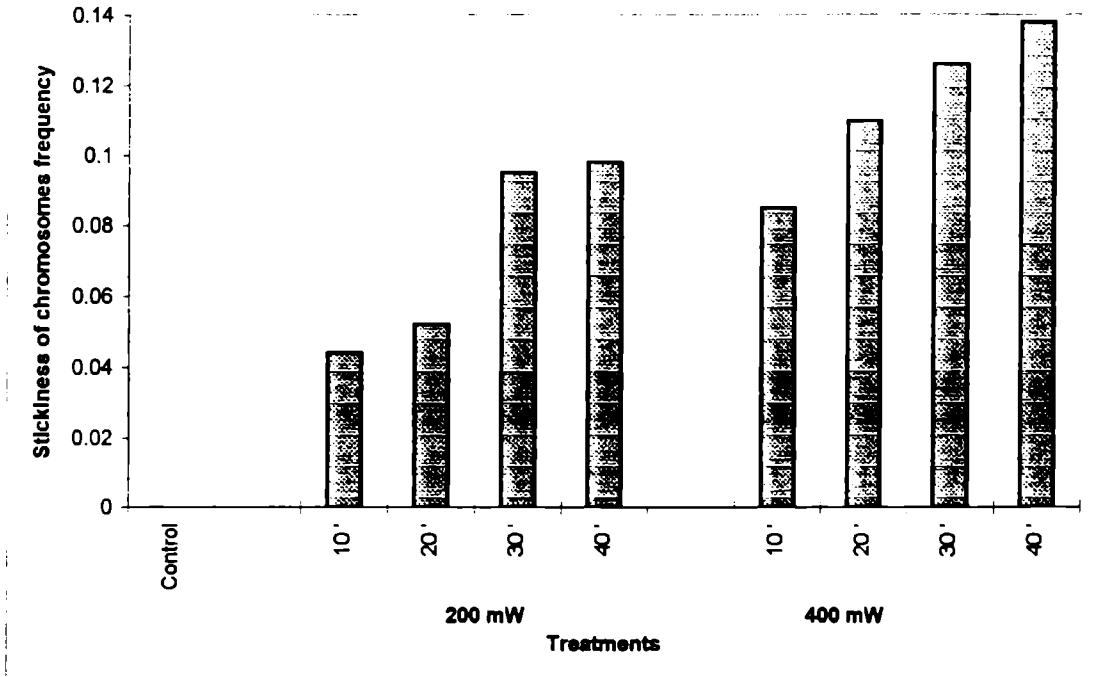


Fig. 9 B : Effect of laser exposure at 488 nm on stickiness of chromosomes in *V. faba*

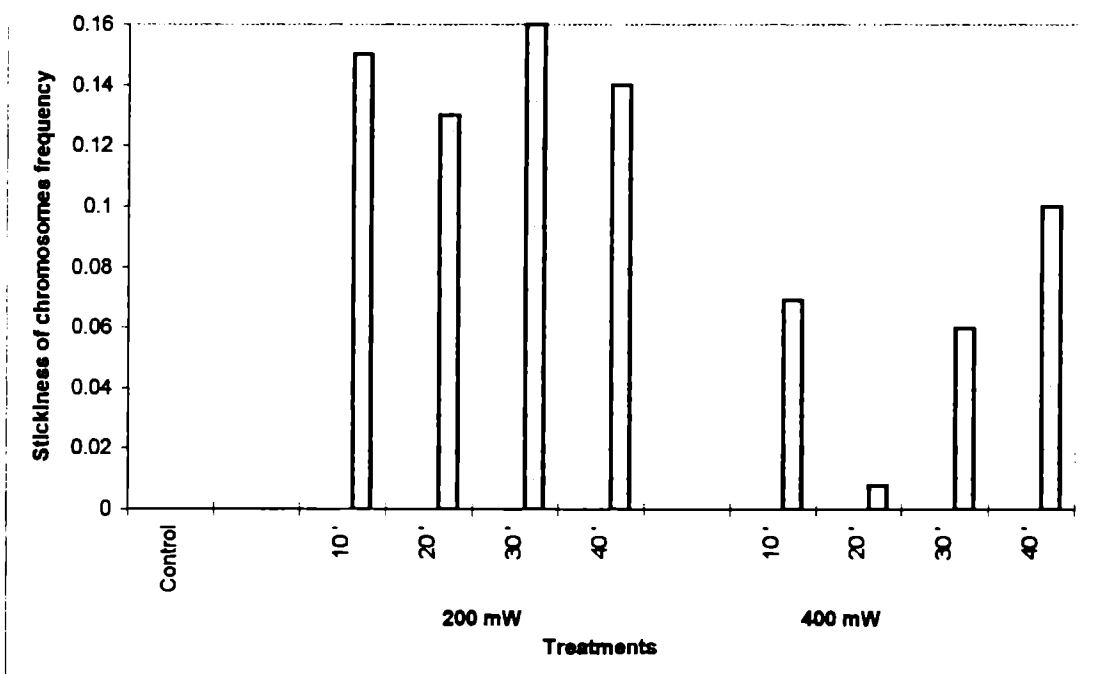


Fig. 10 B : Effect of laser irradiation at 514 nm on stickiness of chromosomes in *V. faba*

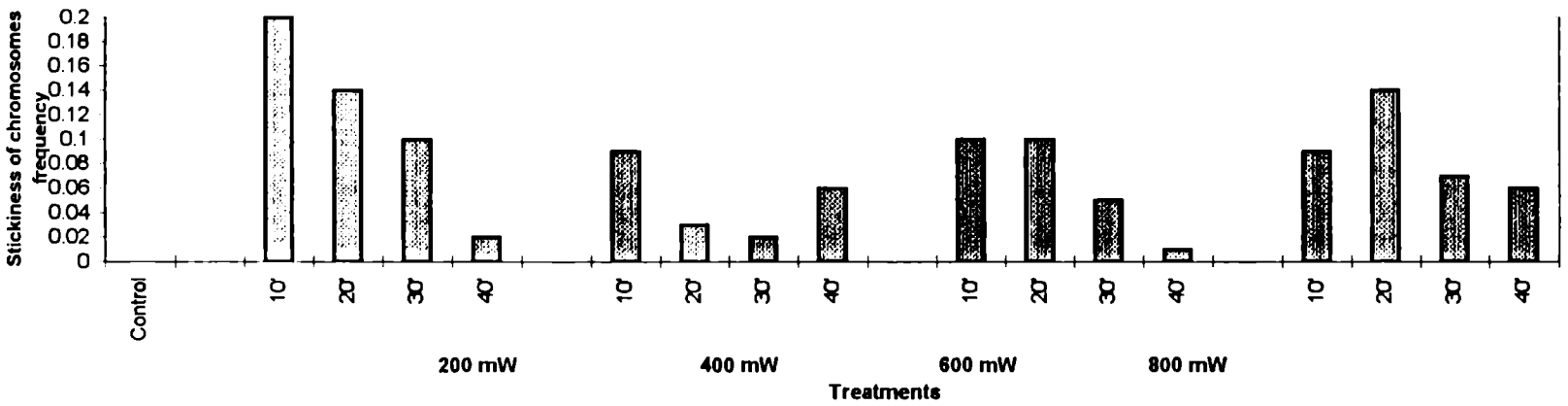


Fig. 11 B : Effect of UV exposure on stickiness of chromosomes in *V. faba*

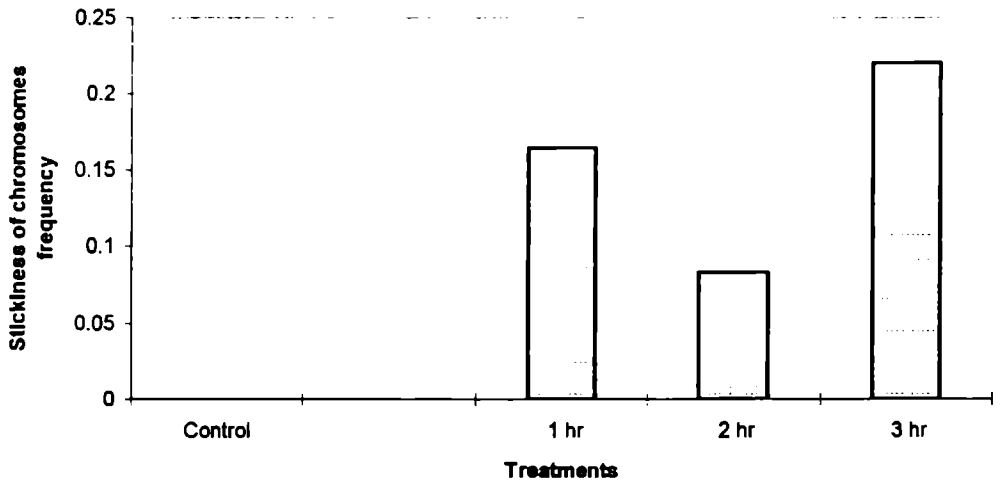


Fig. 12 B : Effect of gamma irradiation on stickiness of chromosomes in *V. faba*

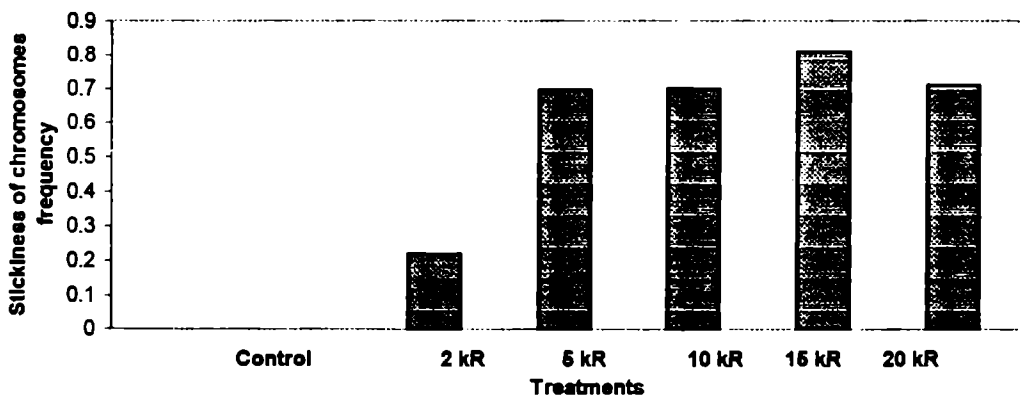


Fig. 13 B : Effect of EMS treatment on stickiness of chromosomes in *V. faba*

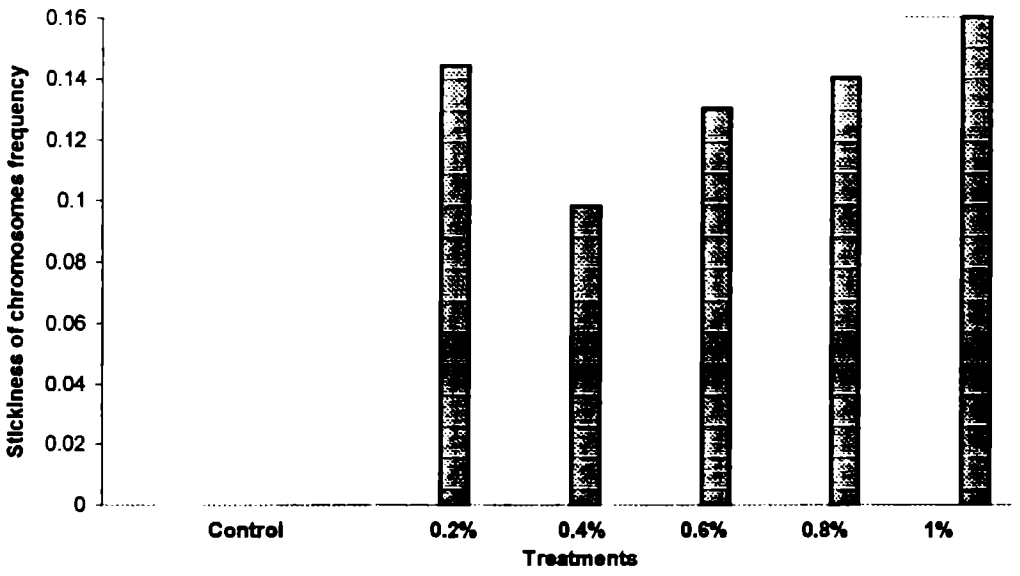
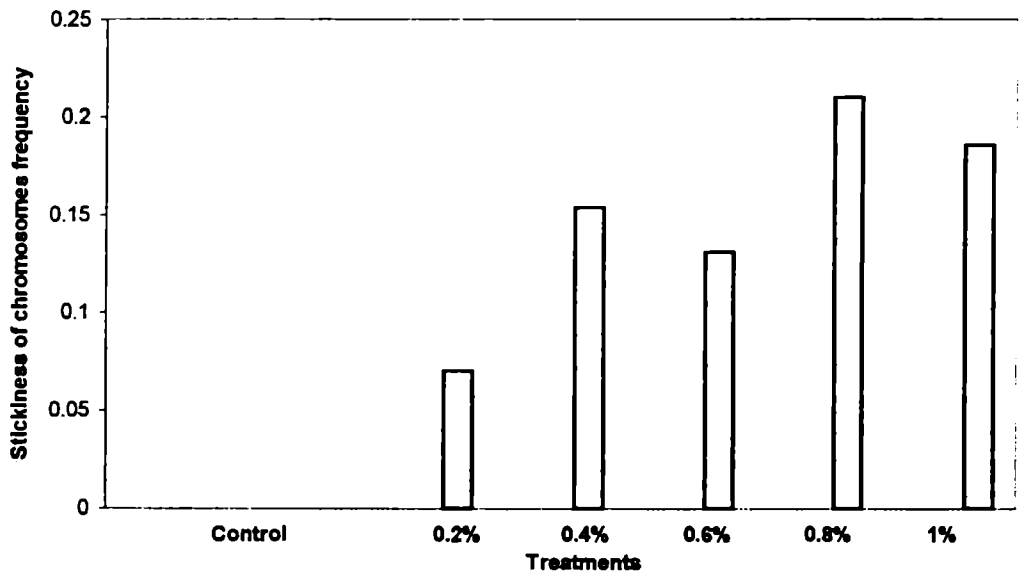


Fig. 14 B : Effect of HA treatment on stickiness of chromosomes in *V. faba*



with increase in concentration (Fig.13B App.VIB). The frequency was similar to that caused by lasers.

In *A. cepa* treated with HA caused dose dependent increase in stickiness frequency with increase in concentration (Fig.12A App.VIA). The stickiness frequency of 476 and 514 nm laser exposures were higher and 488 nm similar to the HA treatment frequency. In *V. faba* also, HA treatments induced an increase in the frequency of this parameter with increase in concentration (Fig.14B App.VIIB). The frequency was higher than that caused by laser exposures.

In *A. cepa* and *V. faba,* lasers at 476 nm caused dose dependent increase and at 488 nm dose dependent decrease in stickiness frequency. Exposures to UV induced dose dependent decrease and treatment with EMS and HA caused dose dependent increase in the frequency of stickiness in both plant species.

4.2.2.1 Nondisjunction of chromosomes

Another common type of aberration observed at metaphase was nondisjunction of chromosomes (plate figs. 2, 18, 49, 50, 67, 82, 90). The frequency of this change was very low in control (untreated) samples of the two plant species (0.09% in *A. cepa* and 0.03% in *V. faba*).

In *A. cepa*, laser exposures of 476 nm induced an increase in nondisjunction frequency with increase in power density (Fig.13A App.IA) although no dose dependence was seen with lasers at 488 nm (Fig.14A App.IIA) and at 514 nm there was a dose dependent decrease in nondisjunction of chromosomes (Fig.15A App.IIIA). Nondisjunction frequencies of 514 nm and

488 nm were higher than that induced at 476 nm. In *V. faba* laser exposures did not produce dose dependent change in nondisjunction frequency (Figs.15B, 16B, 17B App.IB, IIB and IIIB).

Dose dependent increase in nondisjunction frequency with increase in exposure time was observed in *A. cepa* samples exposed to UV irradiation (Fig.16A App.IVA). The frequency of this change induced by UV was lower than that caused by lasers. In *V. faba* no dose dependence in nondisjunction frequency was found in UV exposures (Fig.18B App.IVB). The frequency was similar to that caused by lasers.

Samples of *V. faba* irradiated with γ-rays induced dose dependent increase in nondisjunction frequency with increase in irradiation dose (Fig.19B App.VB) The frequency was similar to that caused by laser exposures.

In *A.cepa*, nondisjunction frequency showed dose dependent increase with increase in EMS concentration (Fig.17A App.VA). The nondisjunction frequency of 476, 488 and high power densities of 514 nm lasers were lower than that of EMS treatment and similar to the lower power densities of 514 nm laser exposures. In *V. faba* also, dose dependent increase in nondisjunction frequency with increase in concentration was observed in EMS treatment (Fig.20B App.VIB). The frequency was several fold higher than that caused by laser exposures.

HA treatments in *A. cepa* induced an increase in nondisjunction of chromosomes with increase in concentration (Fig.18A App.VIA). Nondisjunction induced by lasers of 476, 488 and at 600 and 800 mW of 514nm, were higher in frequency than that induced by HA treatments. In *V. faba* also, HA

Fig. 13 A : Effect of laser irradiation at 476 nm on nondisjunction of chromosomes in *A. cepa*

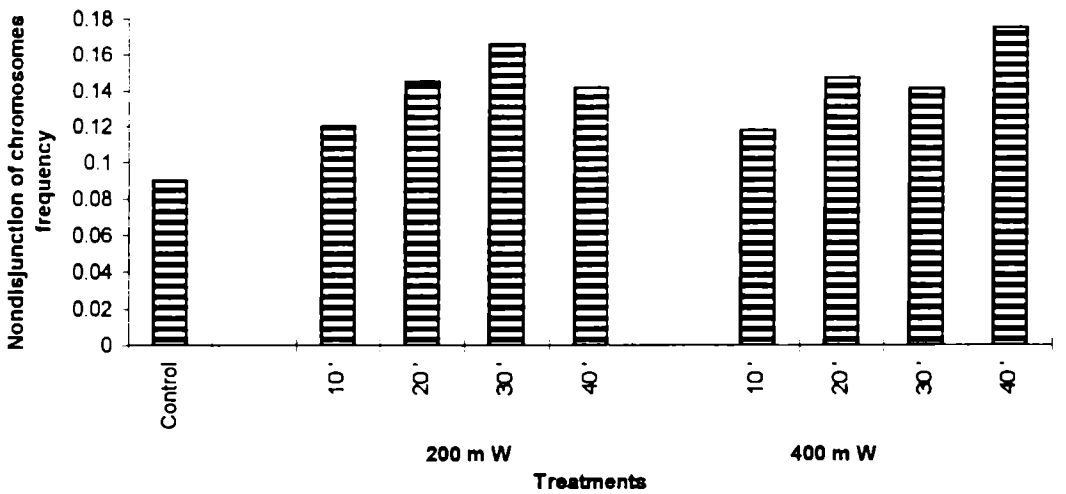


Fig. 14 A : Effect of laser irradiation at 488 nm on nondisjunction of chromosomes in *A. cepa*

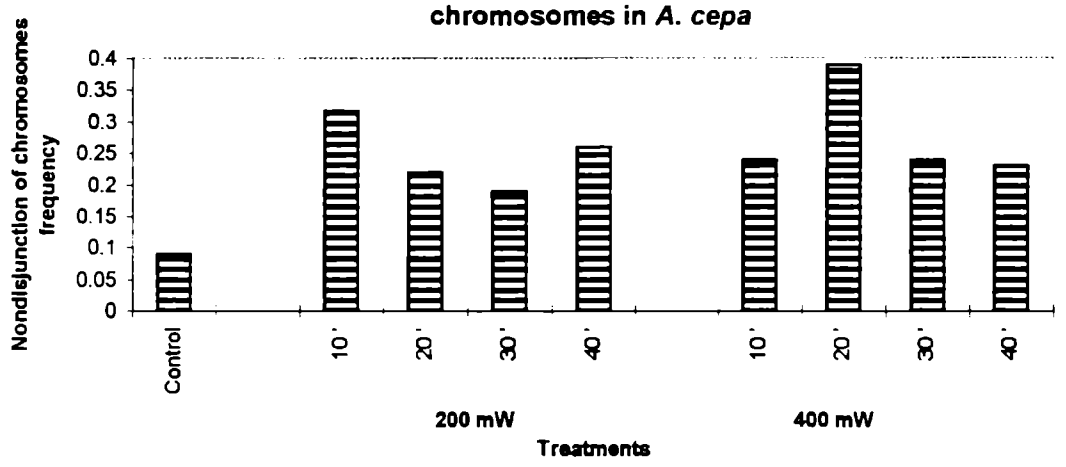


Fig. 15 A : Effect of laser irradiation at 514 nm on nondisjunction of chromosomes in *A. cepa*

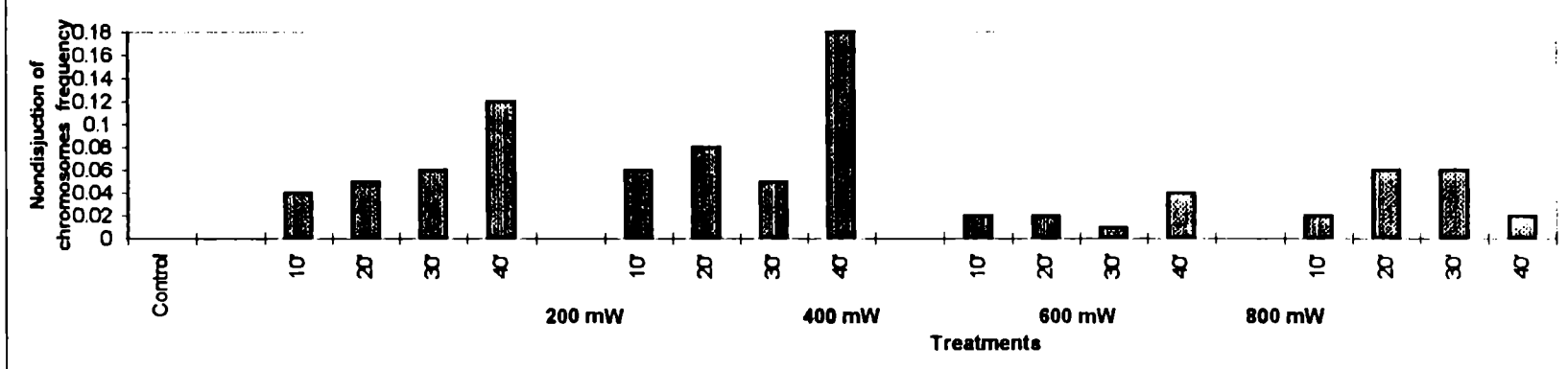


Fig. 16 A : Effect of UV exposures on nondisjunction of chromosomes in *A. cepa*

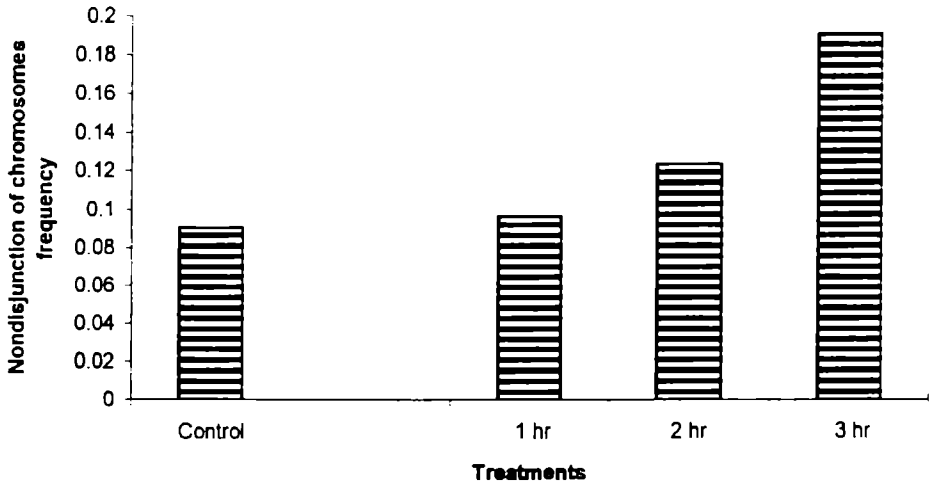


Fig. 17 A : Effect of EMS treatments on nondisjunction of chromosomes in *A. cepa*

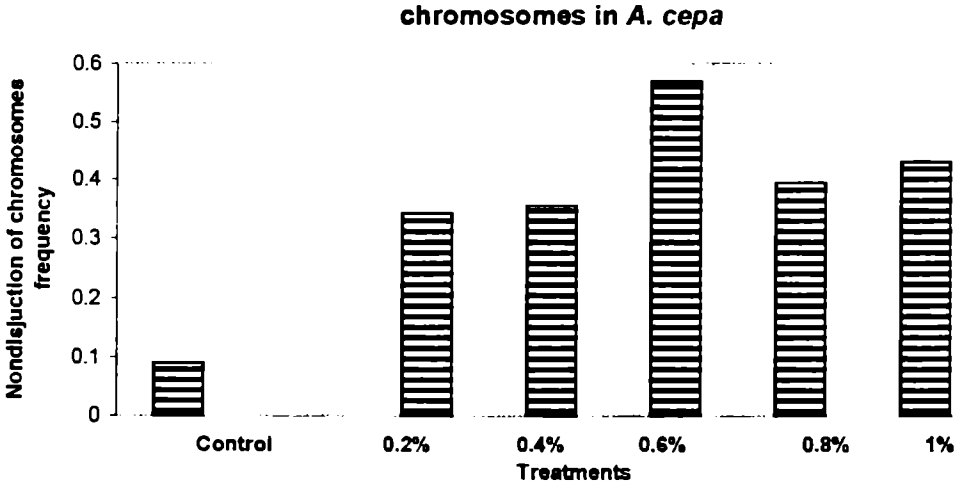


Fig. 18 A : Effect of HA treatments on nondisjunction of chromosomes in *A. cepa*

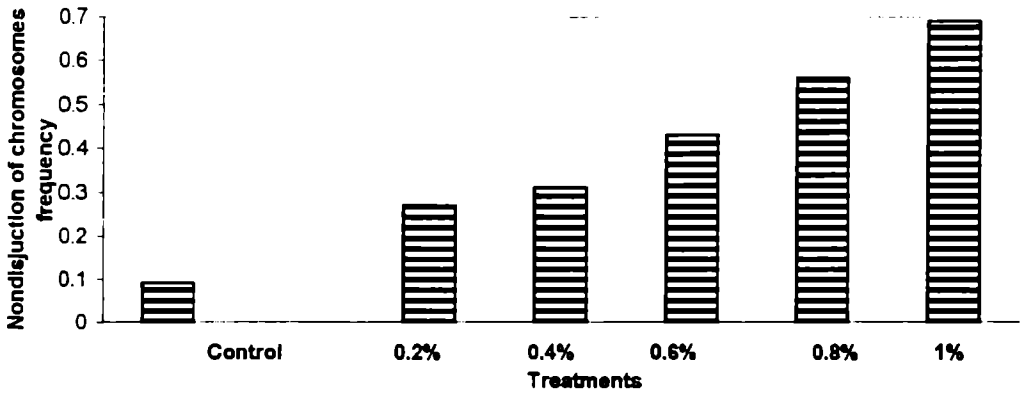


Fig. 15 B : Effect of laser exposure at 476 nm on nondisjunction of chromosomes in *V. faba*

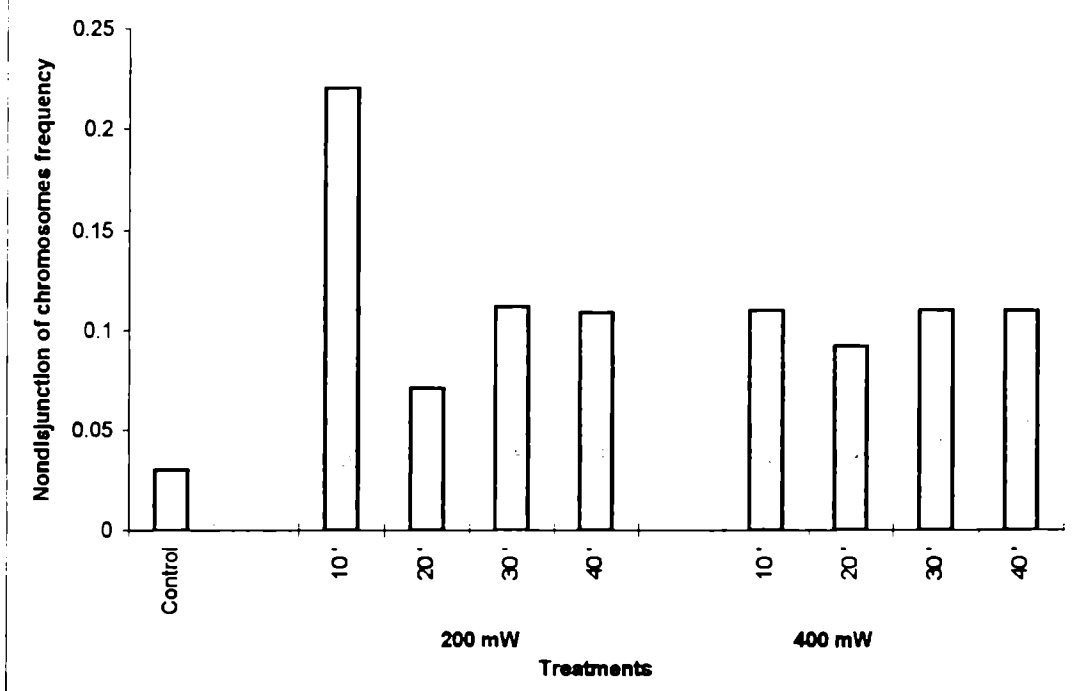


Fig. 16 B : Effect of laser exposure at 488 nm on nondisjunction of chromosomes in *V. faba*

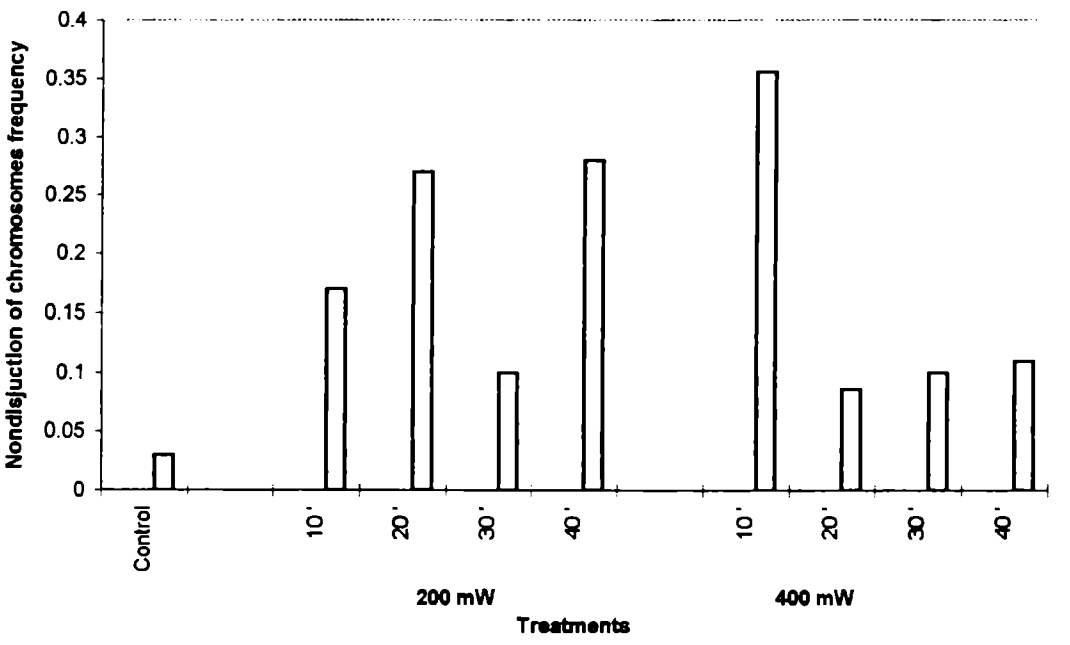


Fig. 17 B : Effect of laser irradiation at 514 nm on nondisjunction of chromosomes in *V. faba*

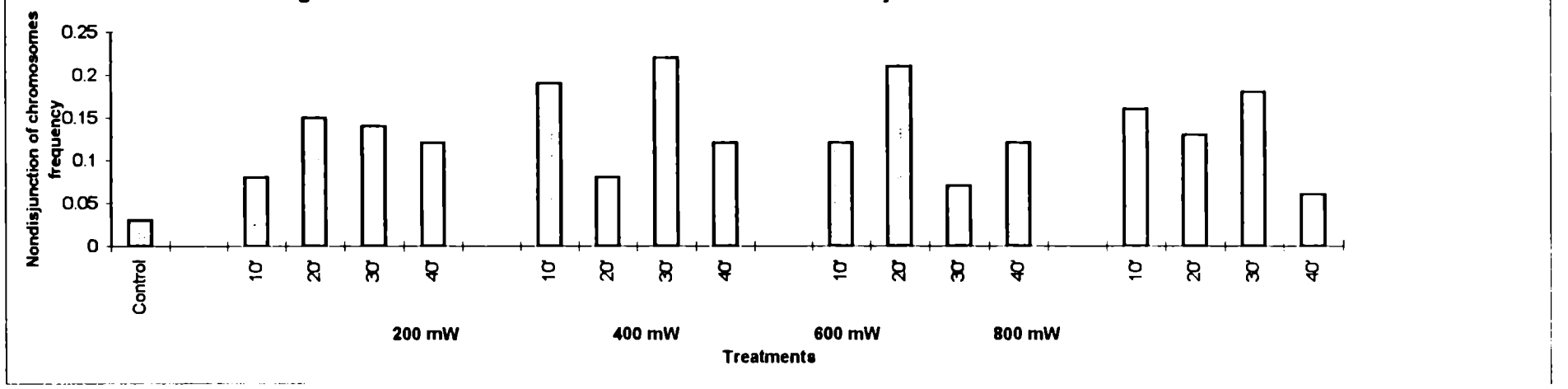


Fig. 18 B : Effect of UV exposure on nondisjunction of chromosomes in *V. faba*

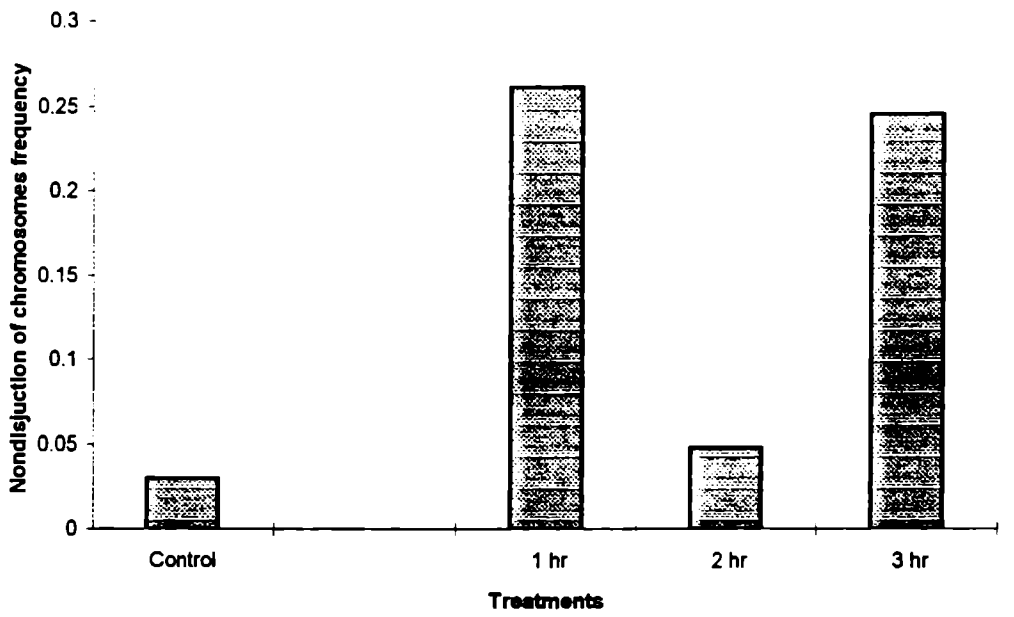


Fig. 19 B : Effect of gamma irradiation on nondisjunction of chromosomes in *V. faba*

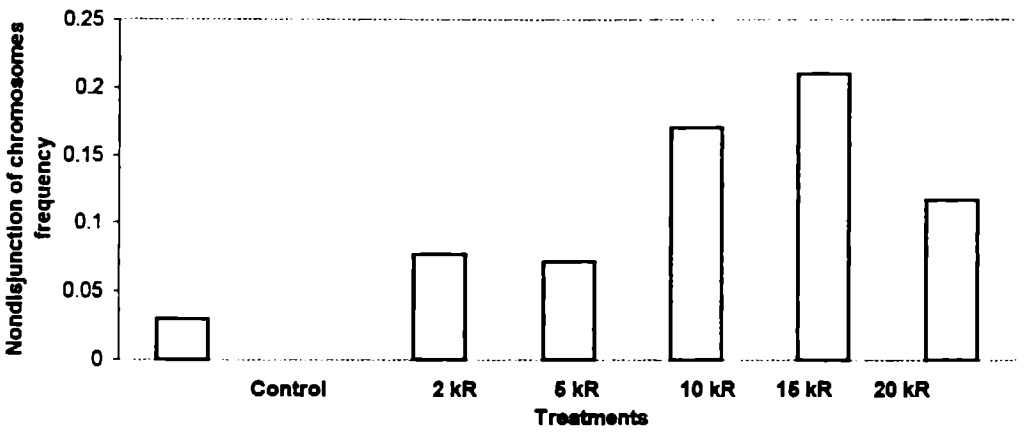


Fig. 20 B : Effect of EMS treatment on nondisjunction of chromosomes in *V. faba*

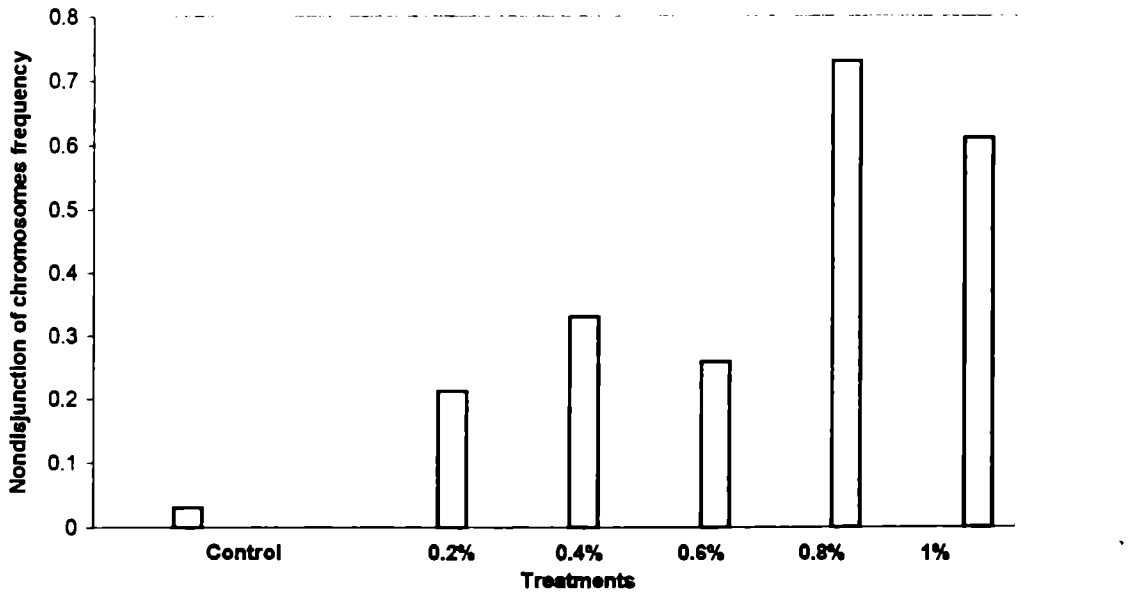
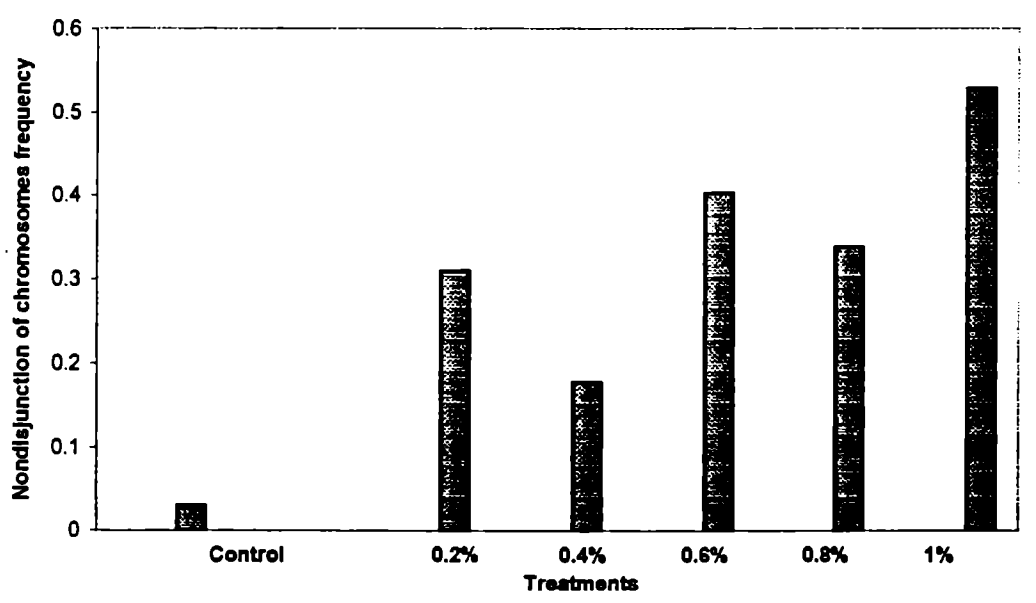


Fig. 21 B : Effect of HA treatment on nondisjunction of chromosomes in *V. faba*



treatment induced an increase in the frequency of this parameter with increase in concentration (Fig.21B App.VIIB). The frequency was higher than that caused by laser exposures.

Lasers caused dose dependent change in nondisjunction of chromosomes in *A. cepa* but not in *V. faba*. While UV exposures caused an increase in the frequency of this parameter in *A. cepa*, no dose dependence could be established in *V. faba.* EMS and HA treatments caused dose dependent increase of nondisjunction with increase in concentrations in both plant species.

4.2.3 Anaphase aberration

4.2.3.1 Bridges

The common type of aberration observed at anaphase in all the treatments were chromosome bridges (plate. figs. 5, 6, 13, 14, 24, 25, 32, 33, 34, 39, 40, 41, 56, 57, 58, 59, 74, 86, 87, 105, 106, 107, 115). Bridges were not found in *A. cepa* control and were present in very low frequency in *V. faba* (0.01%). While no dose dependence could be established for the change in bridge frequency caused by laser exposures at 476 nm in *A. cepa* (Fig.19A App.IA). Laser exposures at 488 and 514 nm caused dose dependent increase in bridge frequency with increase in power density (Figs.20A, 21A App.IIA and IIIA). The frequency of bridges at 476 nm was higher than that induced by 488 and 514 nm laser exposures. In *V. faba* laser exposures induced no dose dependent change in the frequency of bridges but an exposure for 20 min at a power density of 400 mW and wavelength of 488 induced a bridge frequency that was many fold higher than other laser exposures (Figs.2B, 23B and 24B App.IB, IIB, and IIIB).

In *A. cepa* UV caused dose dependent decrease in bridge frequency with increase in exposure time (Fig.22A App.IVA). The bridge frequency elicited by UV was similar to that induced by lasers. In *V. faba*, the frequency of bridges was not dose dependent (Fig.25B App.IVB). The frequency of this parameter induced by UV exposure was lower than that induced by laser exposures.

γ-irradiations in *V. faba* induced a dose dependent increase in bridge frequency with increase in irradiation dose (Fig.26B App.VB). The frequency of bridges in γ-irradiations was higher than that caused by laser exposures.

EMS treatment in *A. cepa* induced dose dependent increase in bridge frequency with increase in treatment concentration (Fig.23A App.VA) and was found to be similar to that induced by laser exposures. In *V. faba* also, bridge frequency showed dose dependent increase with increase in EMS concentration (Fig.27B App.VIB) but was lower than that induced by laser exposures.

HA treatment in *A. cepa* induced no dose dependence in bridges frequency (Fig.24A App.VIA) and was similar to that induced by 488 nm and lower than that of 476 and 514 nm of laser exposures. In *V. faba* also HA treatment induced no dose dependence in bridges frequency (Fig. 28B App. VII B). The frequency of this parameter induced by HA was lower than that induced by 476 nm and similar to 488 and 514 nm of laser exposures.

Fig. 19 A : Effect of laser exposure at 476 nm on bridges in *A. cepa*

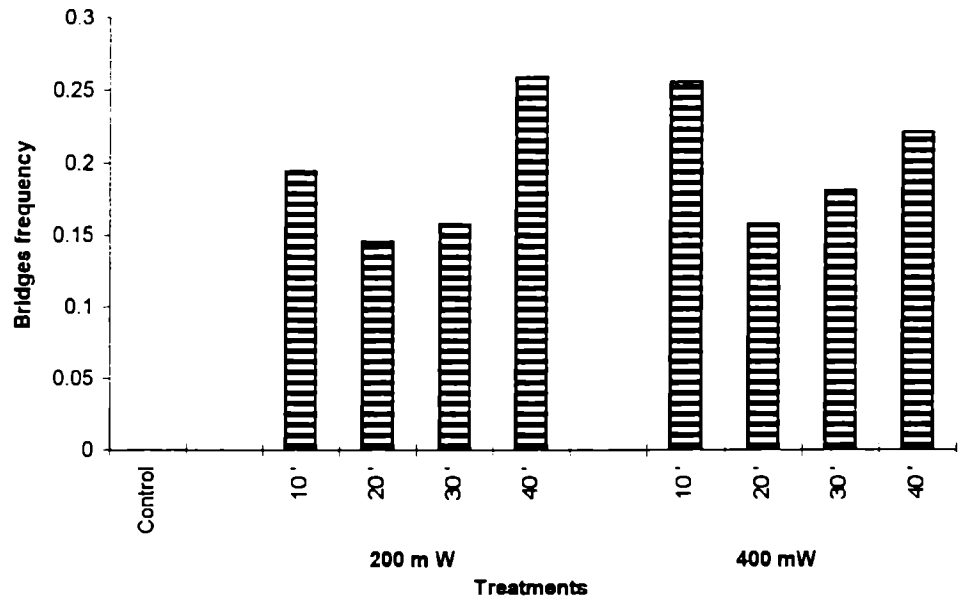


Fig. 20 A : Effect of laser exposure at 488 nm on bridges in *A. cepa*

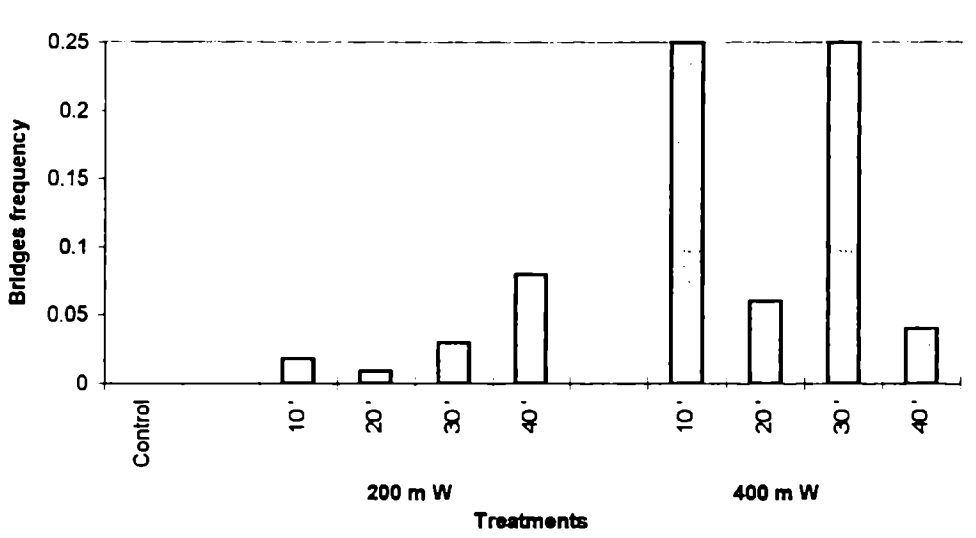


Fig. 21 A : Effect of laser irradiation at 514 nm on bridges in *A. cepa*

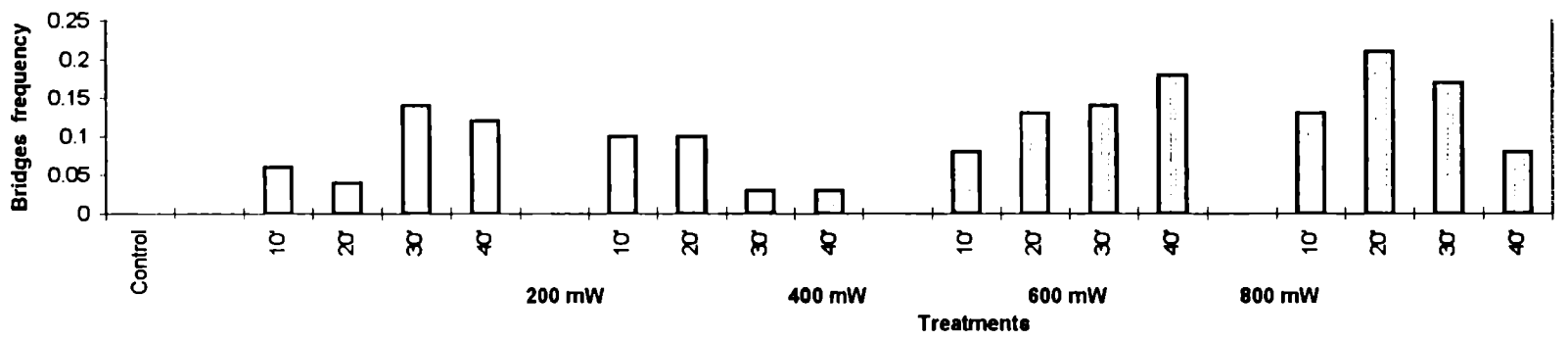


Fig. 22 A : Effect of UV exposure on bridges in *A. cepa*

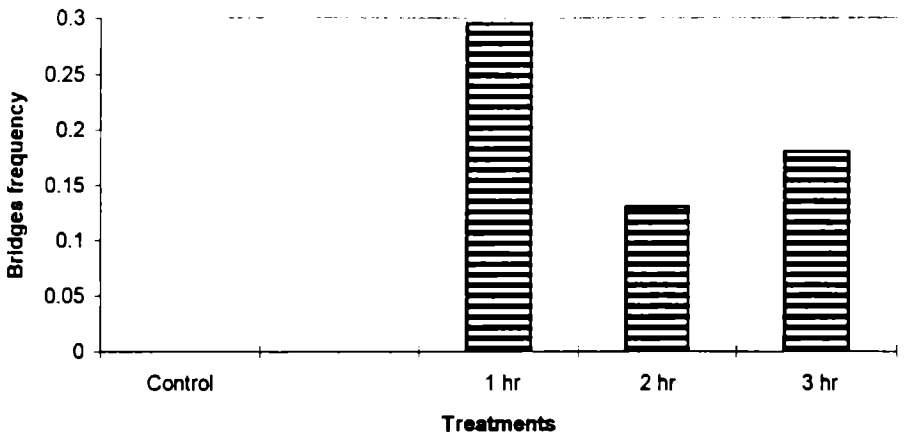


Fig. 23 A : Effect of EMS treatments on bridges in *A. cepa*

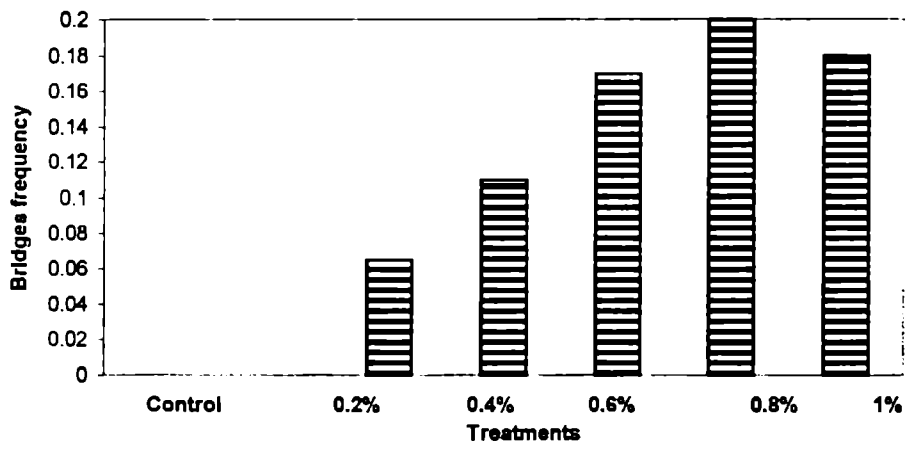


Fig. 24 A : Effect of HA treatments on bridges in *A. cepa*

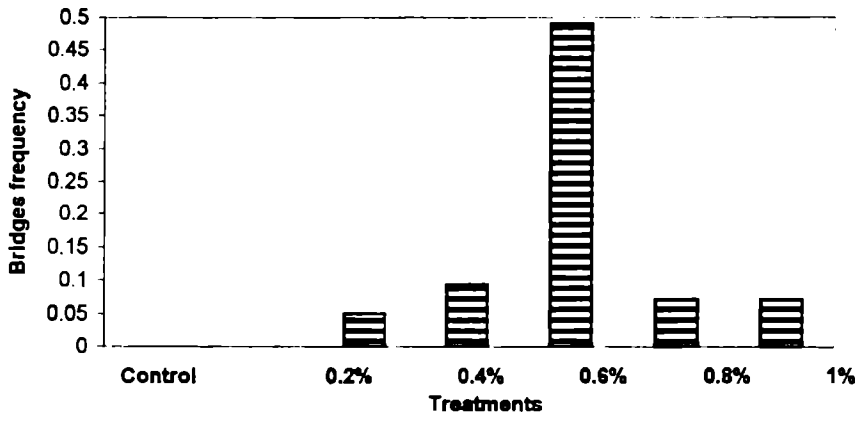


Fig. 22 B : Effect of laser exposure at 476 nm on bridges in *V. faba*

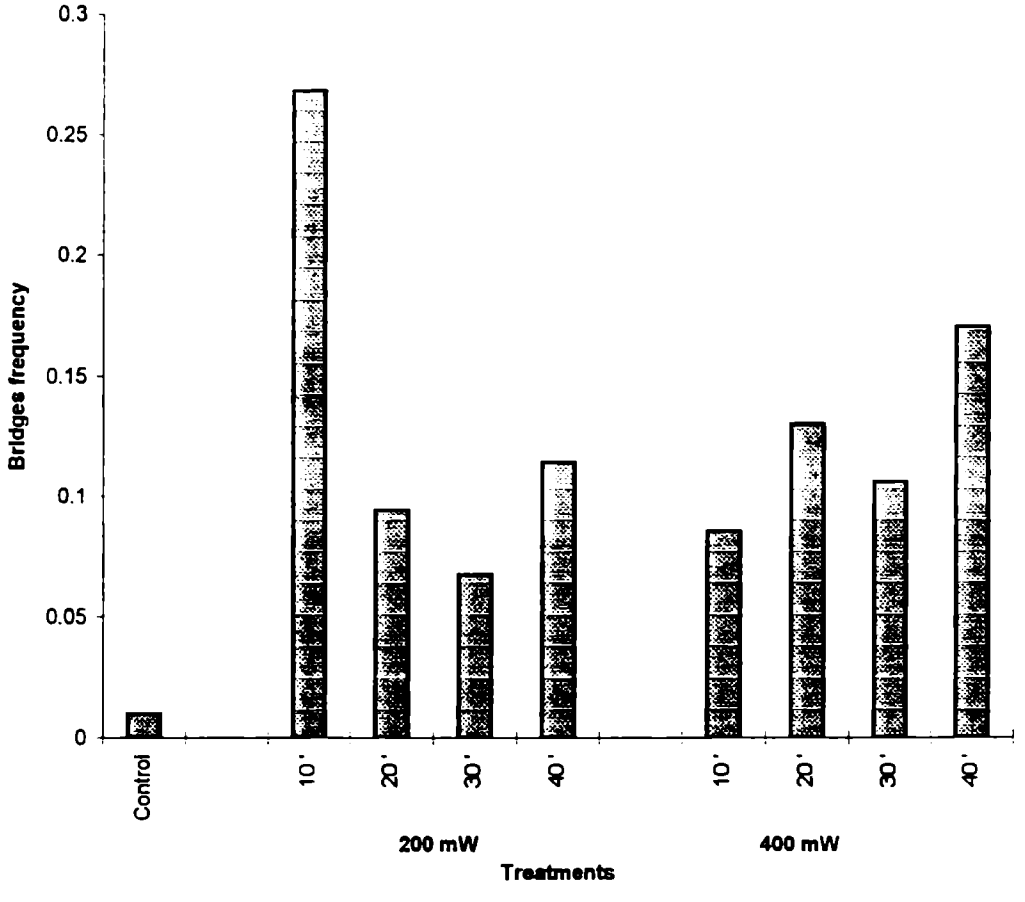


Fig. 23 B : Effect of laser exposure at 488 nm on bridges in *V. faba*

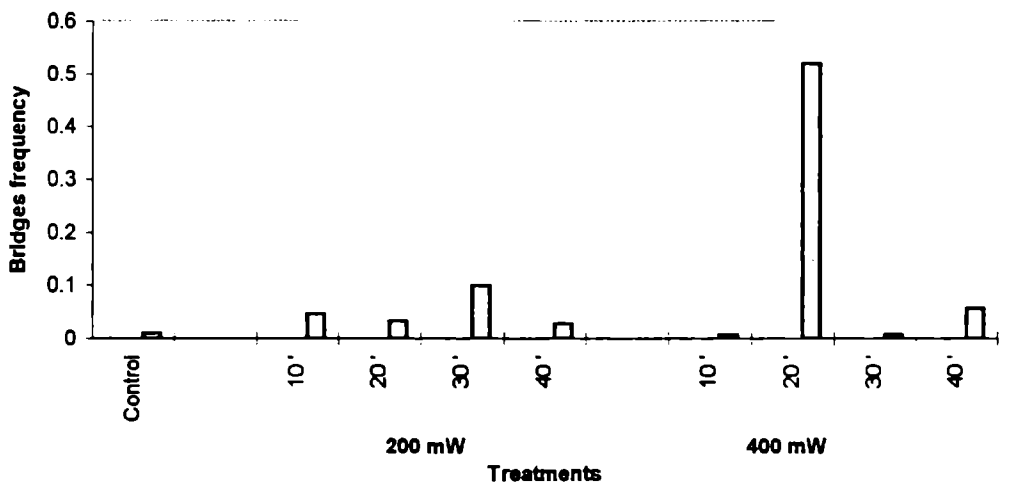


Fig. 24 B : Effect of laser irradiation at 514 nm on bridges in *V. faba*

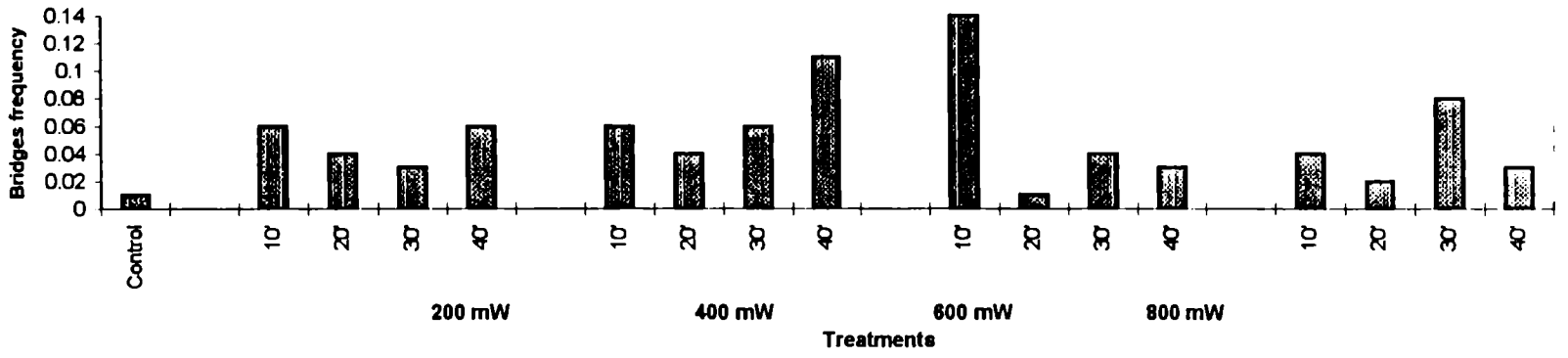


Fig. 25 B : Effect of UV exposure on bridges in *V. faba*

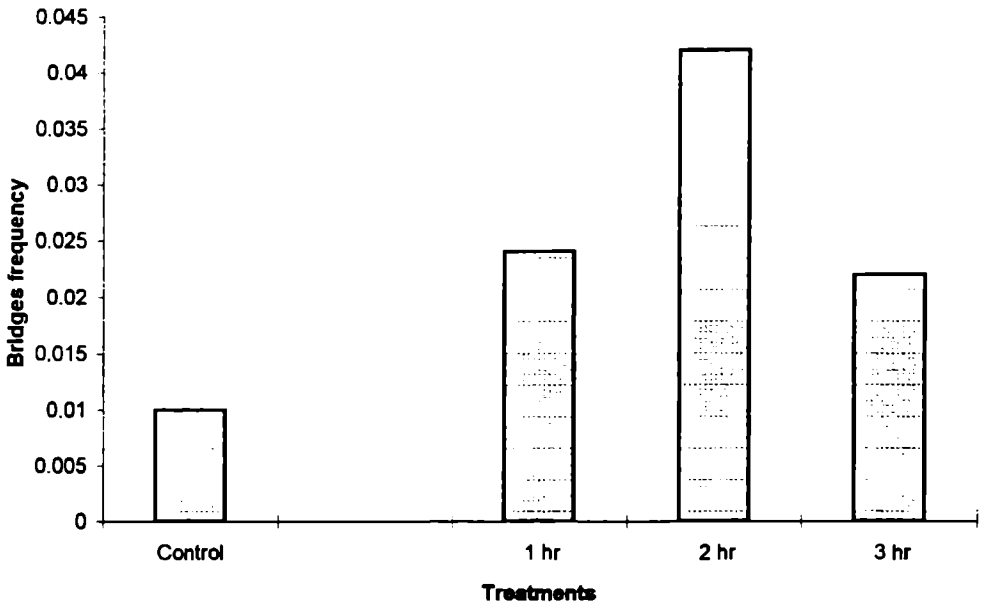


Fig. 26 B : Effect of gamma irradiation on bridges in *V. faba*

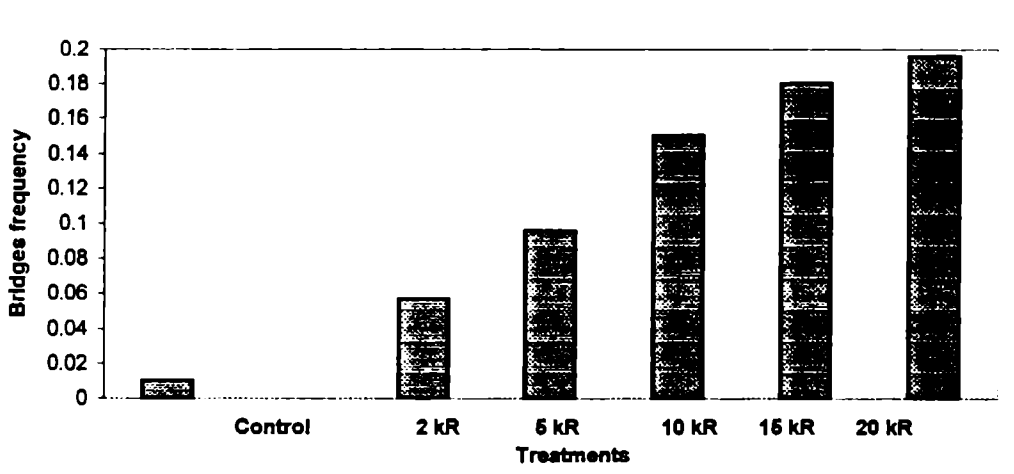


Fig. 27 B : Effect of EMS treatment on bridges in *V. faba*

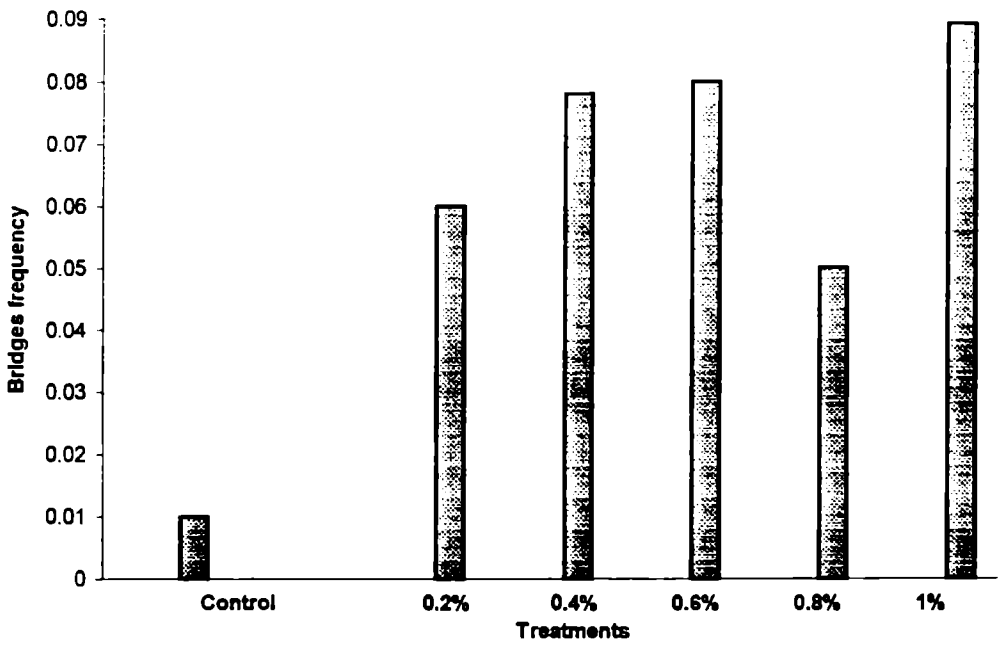
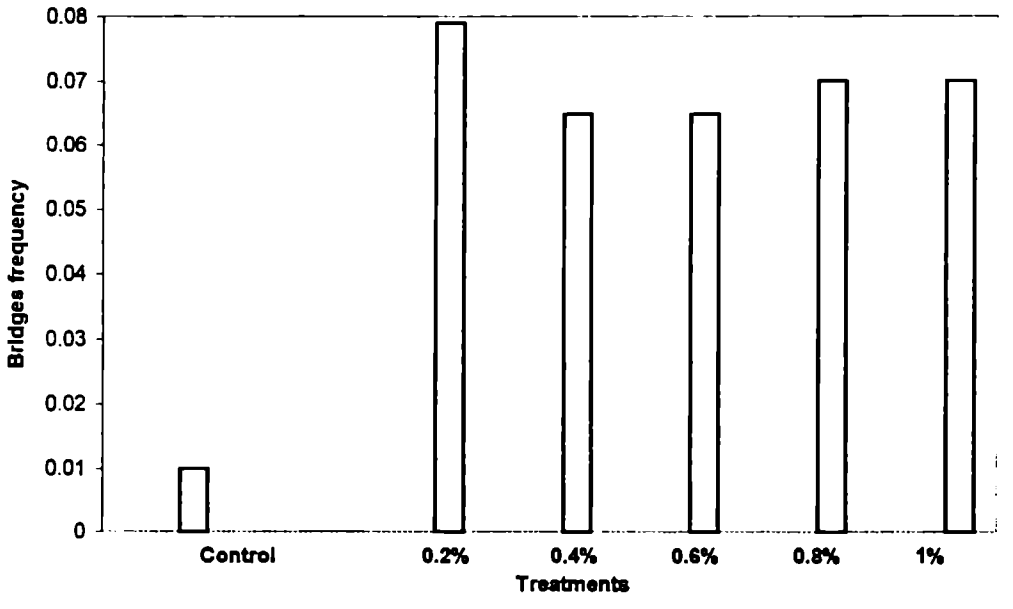


Fig. 28 B : Effect of HA treatment on bridges in *V. faba*



The frequency of bridges induced in *A. cepa* was not dose dependent at 476 nm but showed a dose dependent increase at 488 and 514 nm of laser exposures. Dose dependence was not found in bridges frequencies in *V. faba* exposed to lasers. This parameter in UV exposure showed dose dependent decrease in frequency in *A. cepa* and no dose dependence in *V. faba*. EMS treatment showed dose dependent increase in frequency of this parameter and in HA treatment no dose dependence in both plant species was observed.

4.2.3.2 Laggards

Another type of aberration found at anaphase in low frequencies were chromosomal laggards (plate figs. 6, 14, 33, 35, 39, 40, 41, 57, 58, 59, 86, 105, 106, 107, 114). This was not found in controls of both plant species.

In *A. cepa*, laser exposures induced dose dependent increase in laggard frequency with increase in power density at 476 (Fig.25A App.IA), 488 (Fig.26A App.IIA) and 514 nm (Fig.27A App.IIIA) and the frequencies were similar in all the three wavelengths. In *V. faba,* lasers at 476, 488 and 514 nm did not cause dose dependent change in laggard frequency (Figs.29B, 30B and 31B App.IB, IIB and IIIB) and the frequency of this change induced was similar in these wavelengths.

In *A. cepa*, exposure to UV rays caused no dose dependent change in laggard frequency (Fig.28A App.IVA). The frequency of laggards induced by UV was similar to that induced by lasers. In *V. faba* also, UV did not cause dose dependent change in laggard frequency (Fig.32B App.IVB) and the frequency of laggards was lower than that induced by laser exposures.

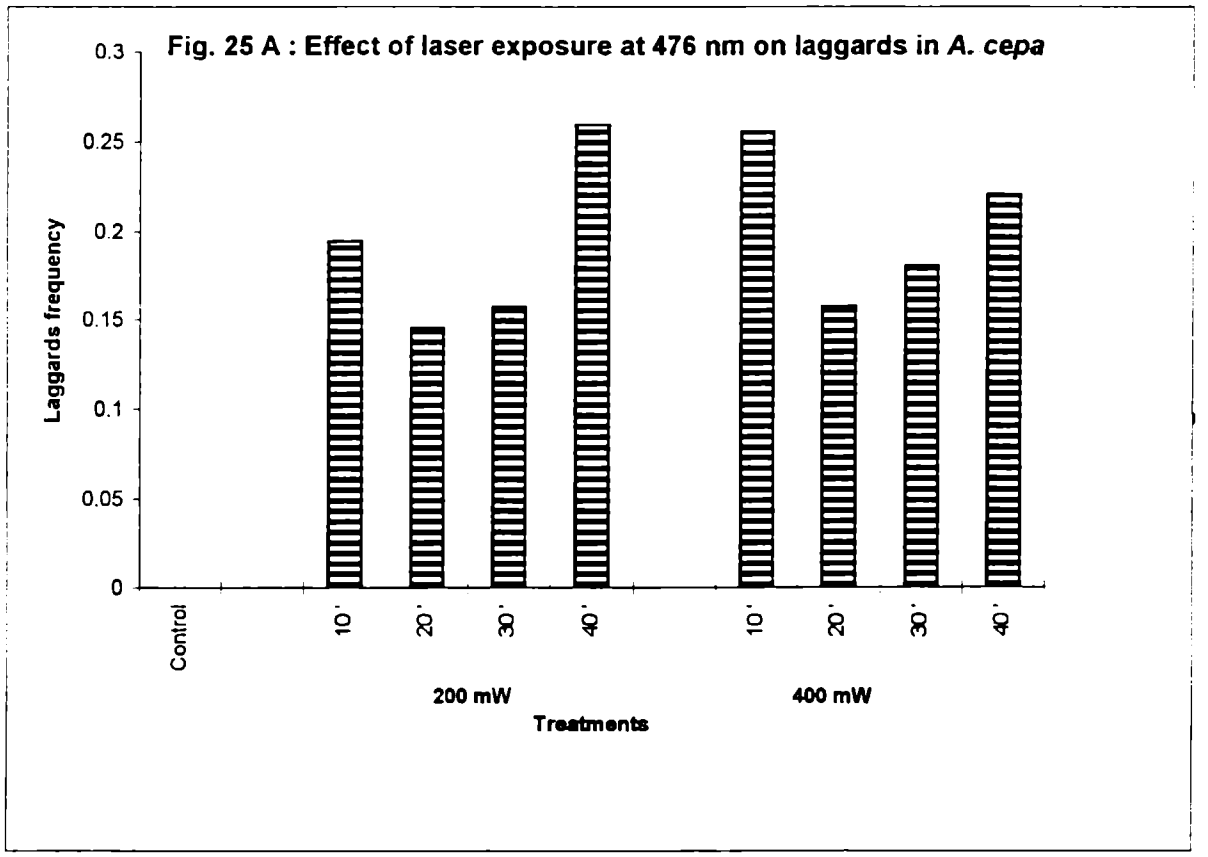


Fig. 25 A : Effect of laser exposure at 476 nm on laggards in A. cepa
Laggards frequency
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
10'
20'
30'
40'
10'
20'
30'
40'
200 mW
400 mW
Treatments

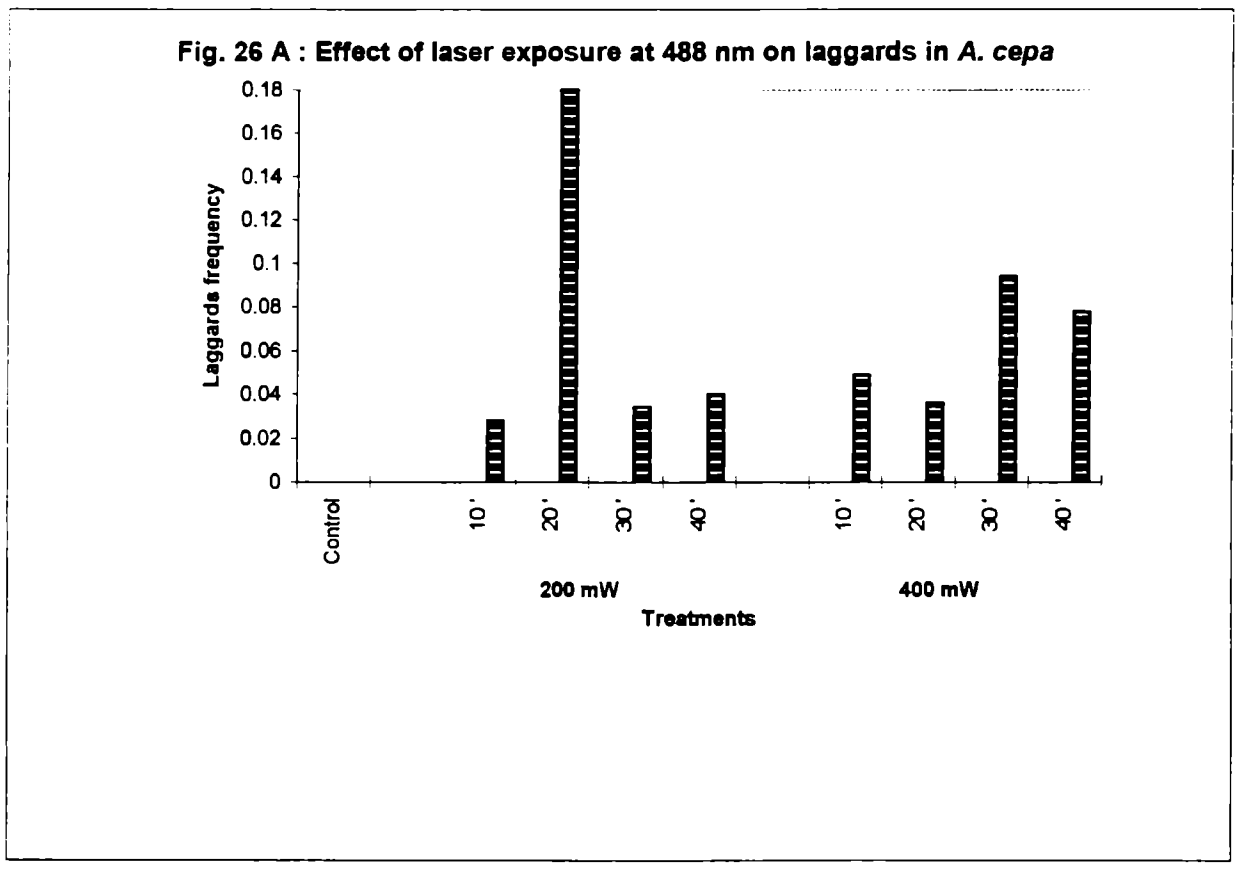


Fig. 26 A : Effect of laser exposure at 488 nm on laggards in A. cepa
Laggards frequency
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Control
10'
20'
30'
40'
10'
20'
30'
40'
200 mW
400 mW
Treatments

Fig. 27 A : Effect of laser irradiation at 514 nm on laggards in *A. cepa*

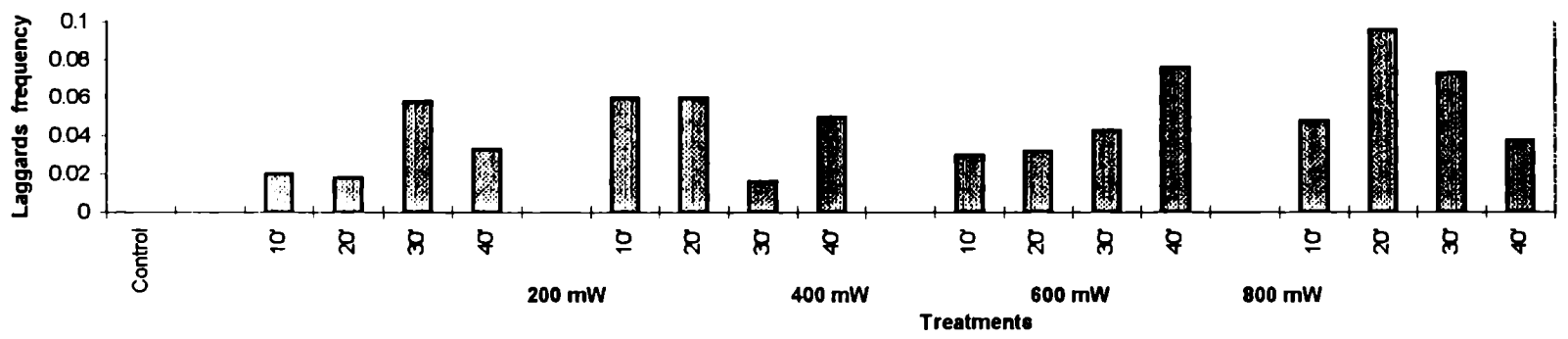


Fig. 28 A : Effect of UV exposure on laggards in *A. cepa*

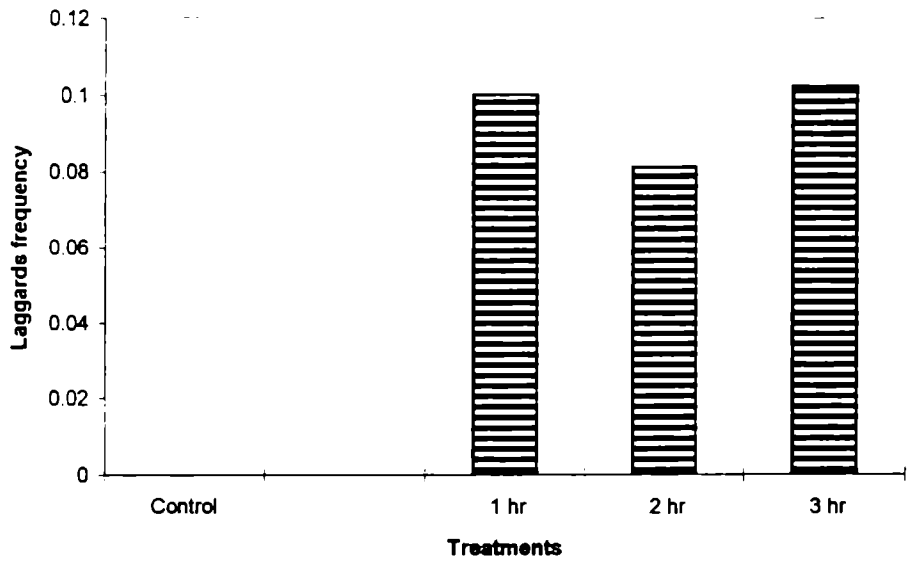


Fig. 29 A : Effect of EMS treatments on laggards in *A. cepa*

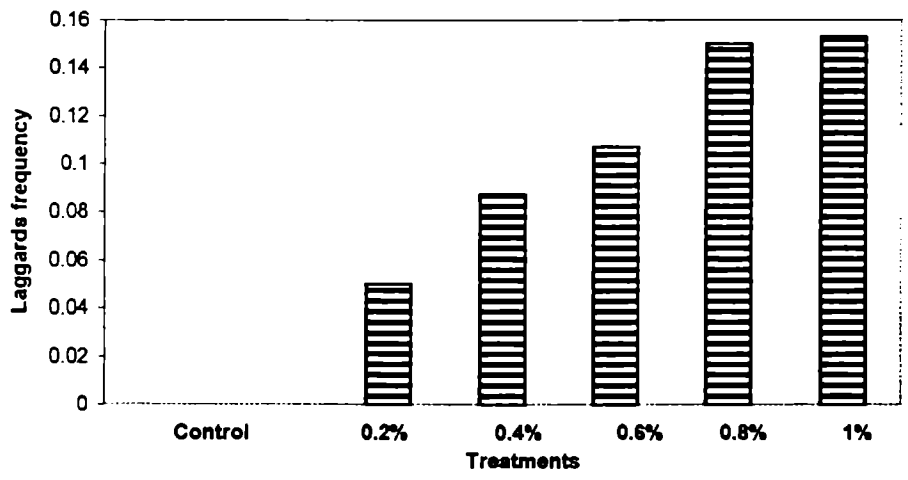


Fig. 30 A : Effect of HA treatments on laggards in *A. cepa*

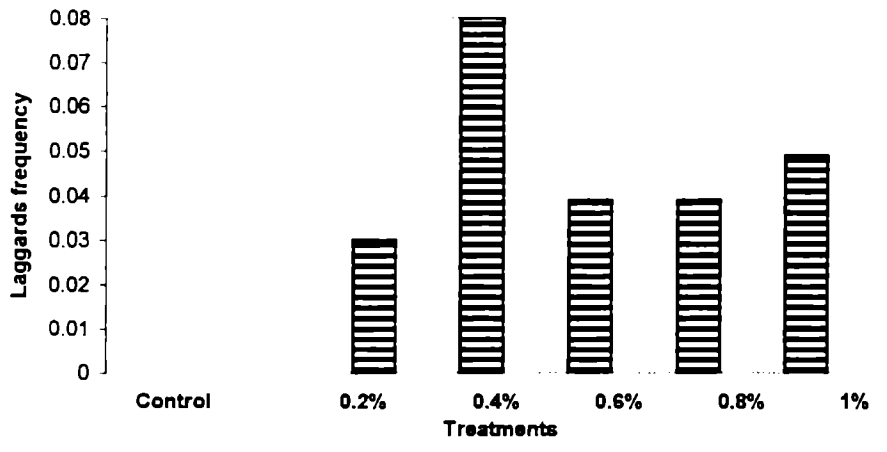


Fig. 29 B : Effect of laser exposure at 476 nm on laggards in *V. faba*

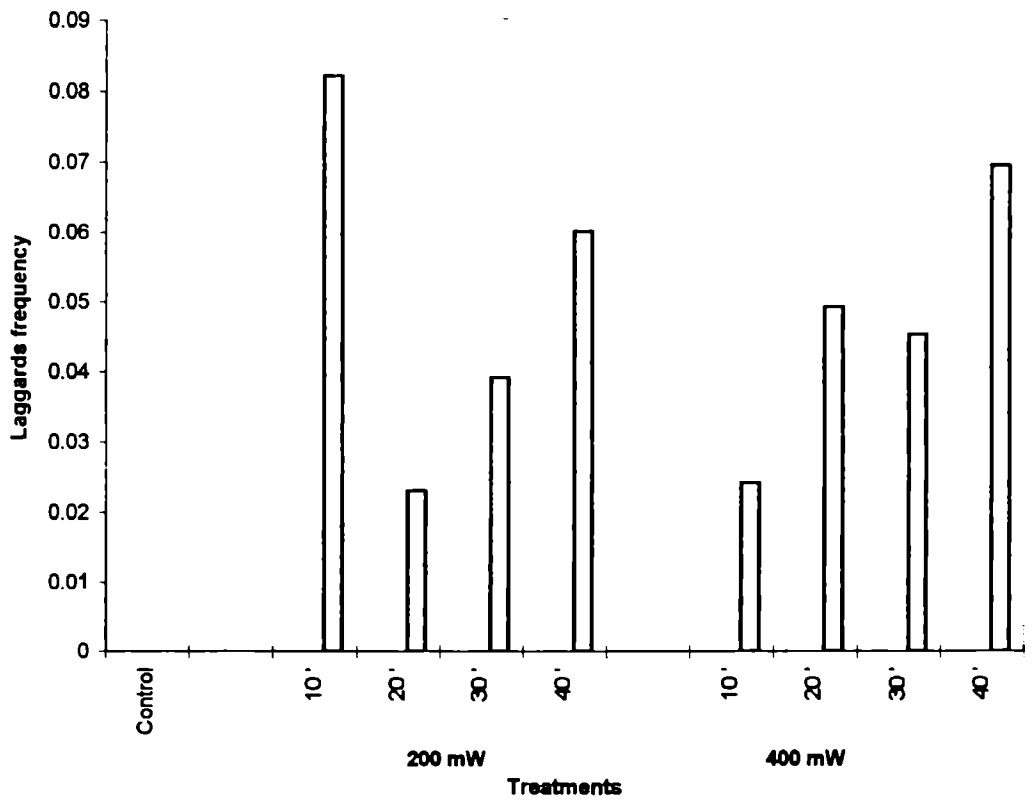


Fig. 30 B : Effect of laser exposure at 488 nm on laggards in *V. faba*

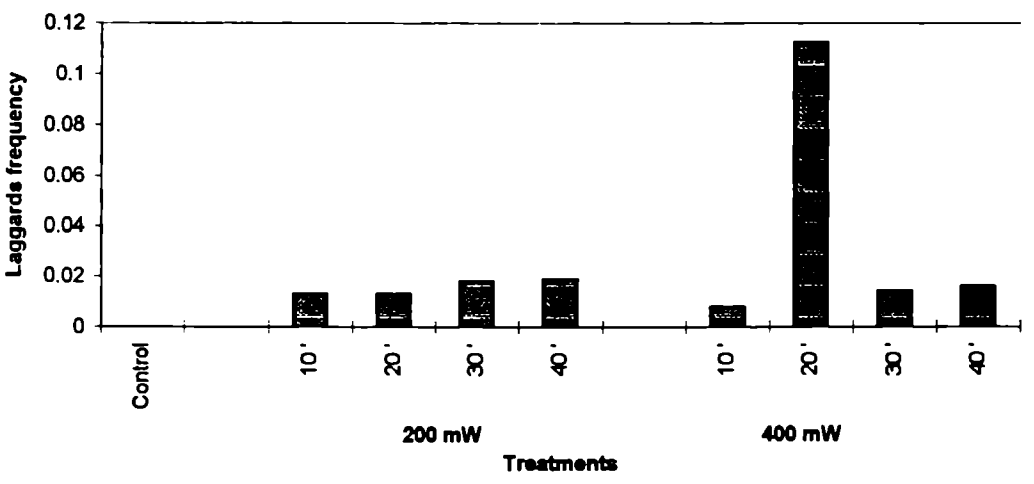


Fig. 31 B : Effect of laser irradiation at 514 nm on laggards in *V. faba*

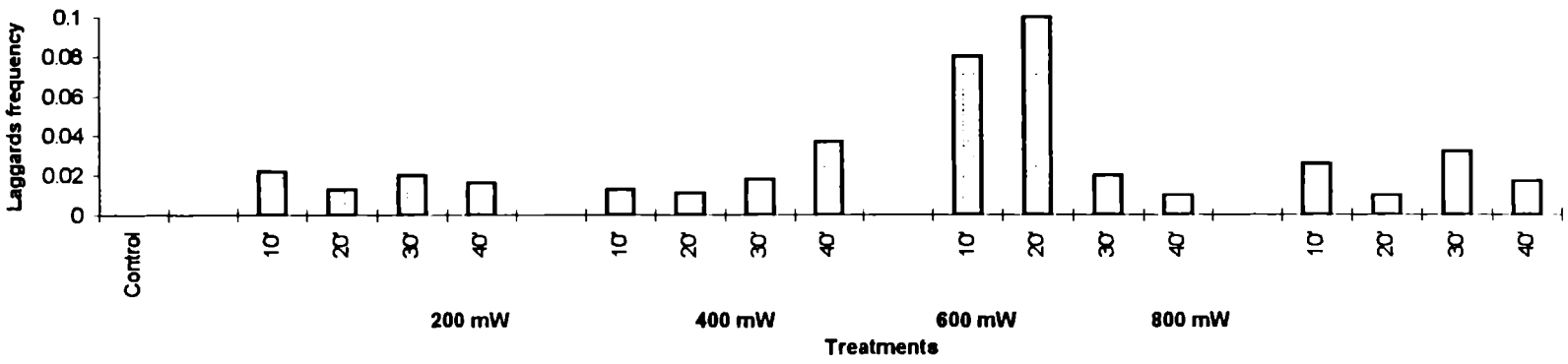


Fig. 32 B : Effect of UV exposure on laggards in *V. faba*

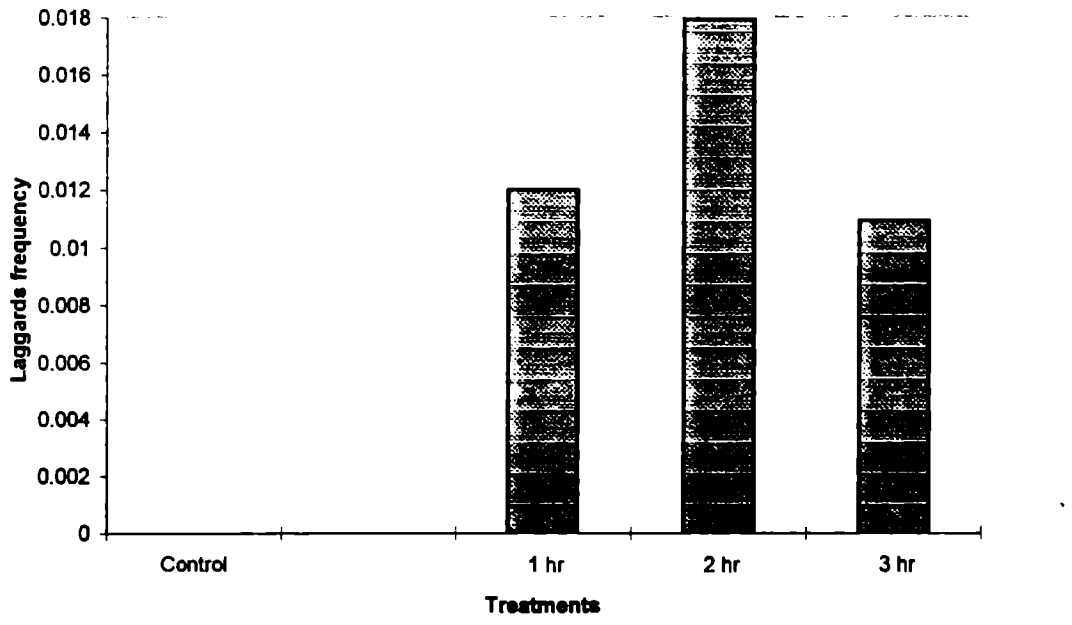


Fig. 33 B : Effect of gamma irradiation on laggards in *V. faba*

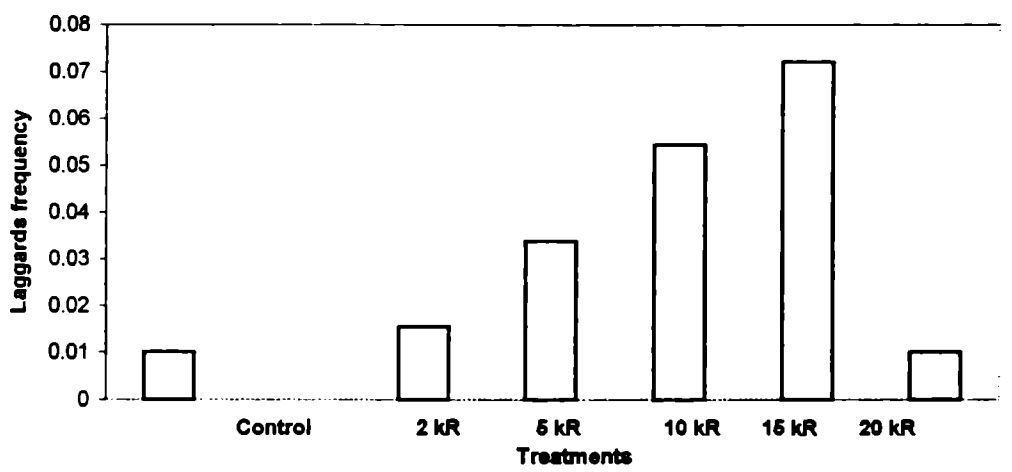


Fig. 34 B : Effect of EMS treatment on laggards in *V. faba*

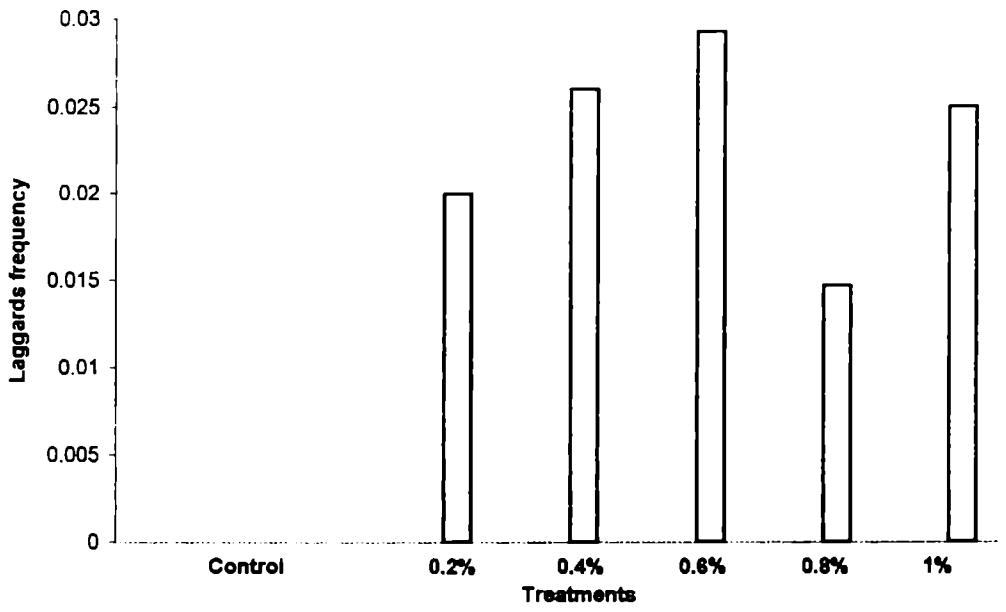
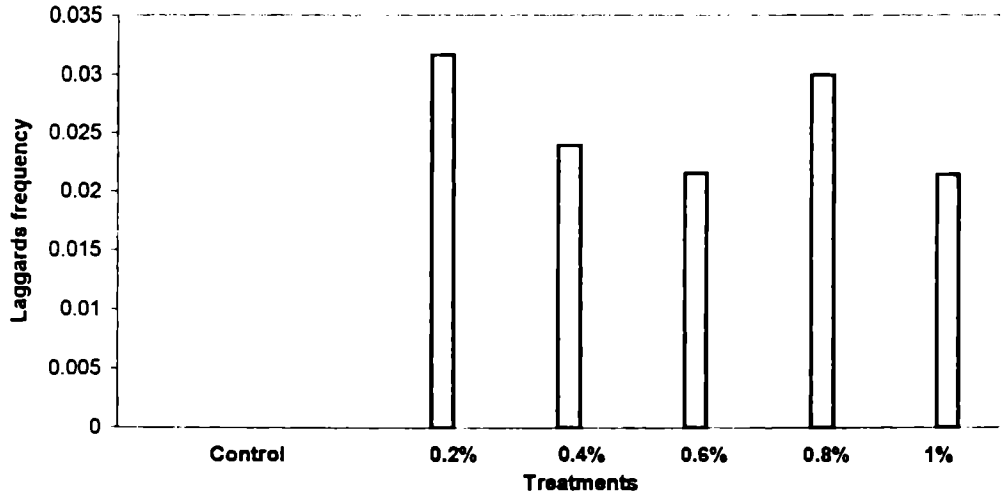


Fig. 35 B : Effect of HA treatment on laggards in *V. faba*



γ-irradations in *V. faba* induced dose dependent increase in laggard frequency with increase in power density (Fig.33B App.VB). The frequency of this parameter was similar to that induced by laser exposures.

In *A. cepa*, EMS treatment induced dose dependent increase in laggard frequency with increase in EMS concentration (Fig.29A App.VA) and the frequency was higher than that of laser exposures. In *V. faba*, EMS did not cause dose dependent change in laggard frequency (Fig.34B App.VIB) and the frequency of this parameter was similar to that induced by laser exposures.

HA treatment in *A. cepa* caused no dose dependent change in laggard frequency (Fig.30A App.VIA) and it was similar to that induced by laser exposures. The same results were observed with *V. faba* also (Fig.35B App.VIIB).

Laggard frequency in laser exposures and EMS treatments showed dose dependent increase in *A. cepa* but failed to show dose dependence in *V. faba*. UV exposures and HA treatments caused no dose dependent change in laggard frequency in both plant species.

4.2.4 Telophase aberration

4.2.4.1 Micronucleate cells

One of the aberration observed at telophase was cells with micronuclei (Plate figs.17, 28, 62, 77, 110, 111, 112). It was not observed in control and many of the treatments in *A. cepa* and were encountered in control and many of the treatments in *V. faba*.

In *A. cepa*, cells with micronuclei were seen in very low frequency in some of the laser exposures (Figs.31A, 32A and 33A App.IA, IIA and IIIA) and was not dose dependent. In *V. faba*, laser exposure at 476 nm induced micronucleate cell formation in very low frequency (Fig.36B App.IB) although 488 nm of lasers exposure caused dose dependent increase in micronucleate cell frequency with increase in power density (Fig.37B App.IIB) and 514 nm laser exposure did not cause dose dependence in micronucleate cells frequency except in exposures for 10 min at power density 600 mW, the frequency of which was several fold higher to the other laser treatments (Fig.38B App.IIIB). The frequency of this parameter induced by 476 nm was several fold lower than that caused by 488 and 514 nm.

In *A. cepa,* UV exposure caused dose dependent decrease in frequency of this parameter and was not seen at 3 hours of UV exposure (Fig.34A App.IVA). The frequency was similar to that of laser exposures. In *V. faba*, UV caused micronucleate cell formation only in exposures of 2 hours (Fig.39B App.IVB) and the frequency was higher than that induced by 476 nm and lower than 488 and 514 nm of laser exposures.

γ-irradiations in *V. faba* induced dose dependent increase in micronucleate cell frequency with increase in irradiation dose (Fig.40B App.VB). The frequency of micronucleate cells was several fold higher to that induced by laser exposures.

EMS treatment in *A. cepa* caused micronucleate cell formation only in one dose (Fig.35A App.VA) and the frequency was lower to that induced by lasers. In *V. faba,* EMS did not cause dose dependent change in the frequency of micronucleate cells (Fig.41B App.VIB) and the frequency was

Fig. 31 A : Effect of laser exposure at 476 nm on micronucleate cells in *A. cepa*

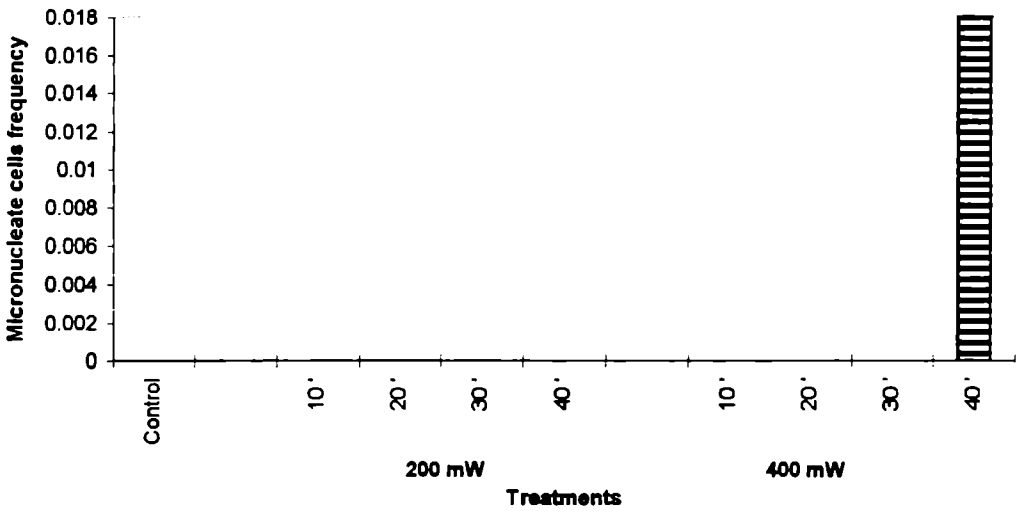


Fig. 32 A : Effect of laser exposure at 488 nm on micronucleate cells in *A. cepa*

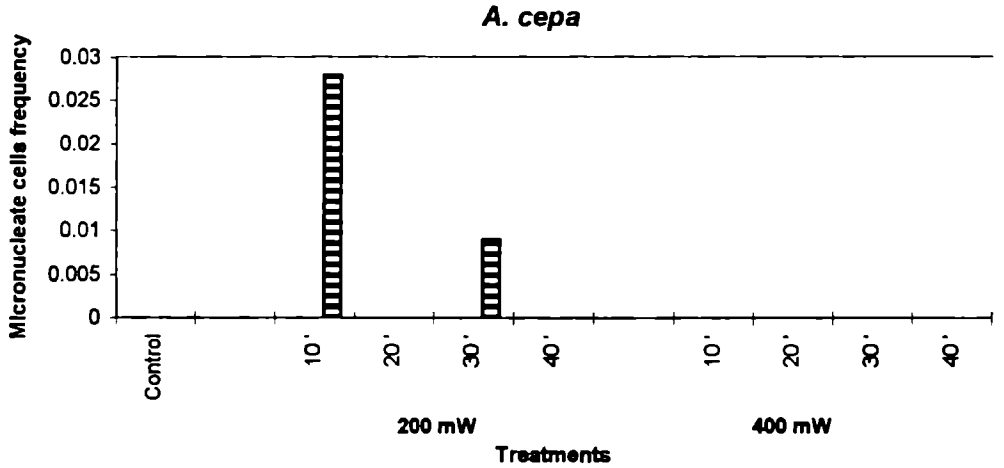


Fig. 33 A : Effect of laser irradiation at 514 nm on micronucleate cells in *A. cepa*

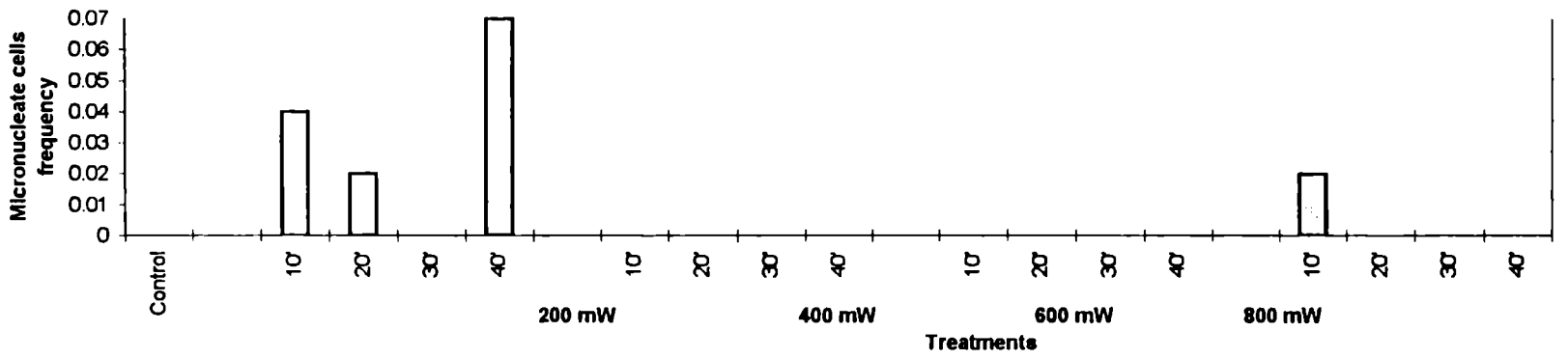


Fig. 34 A : Effect of UV exposure on micronucleate cells in *A. cepa*

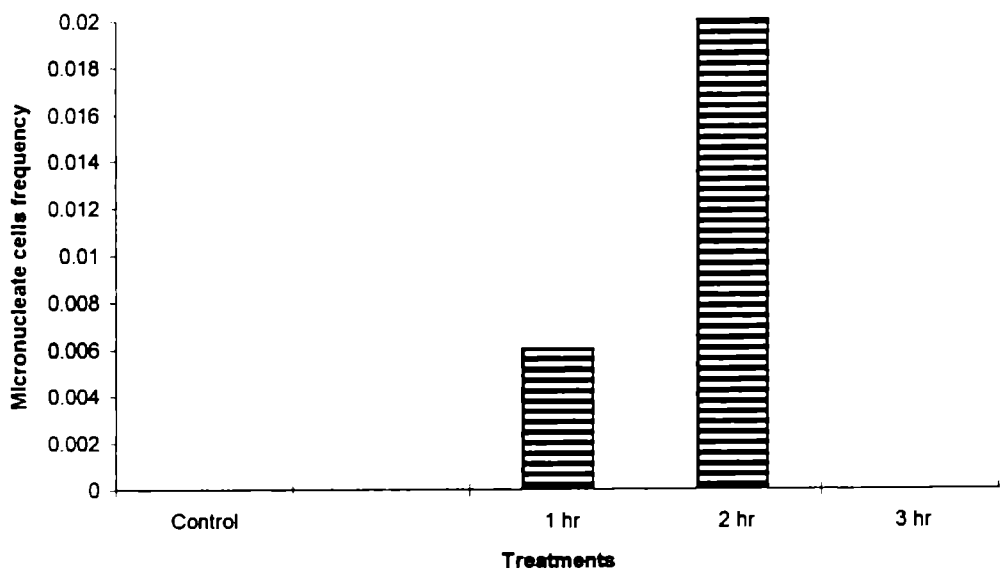


Fig. 35 A : Effect of EMS treatments on micronucleate cells in *A. cepa*

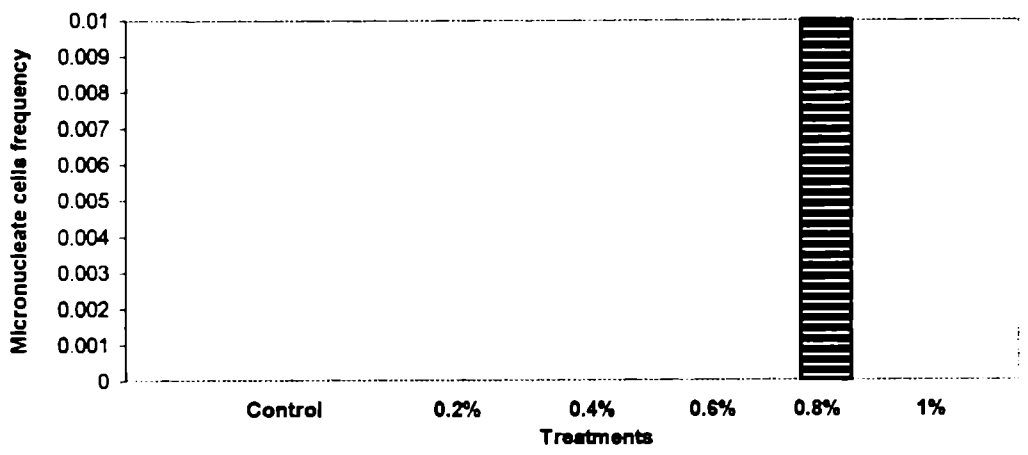


Fig. 36 A : Effect of HA treatment on micronucleate cells in *A. cepa*

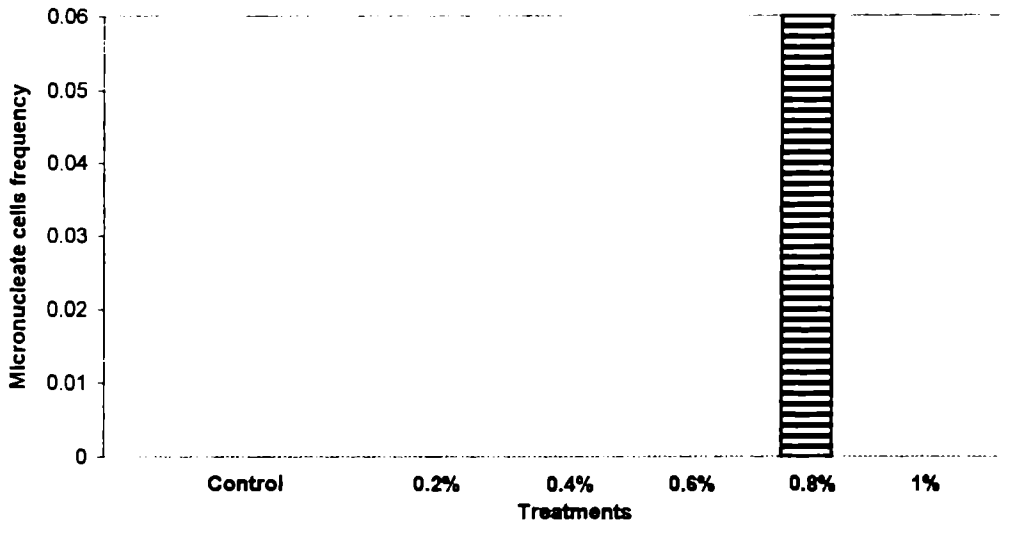


Fig. 36 B : Effect of laser exposure at 476 nm on micronucleate cells in *V. faba*

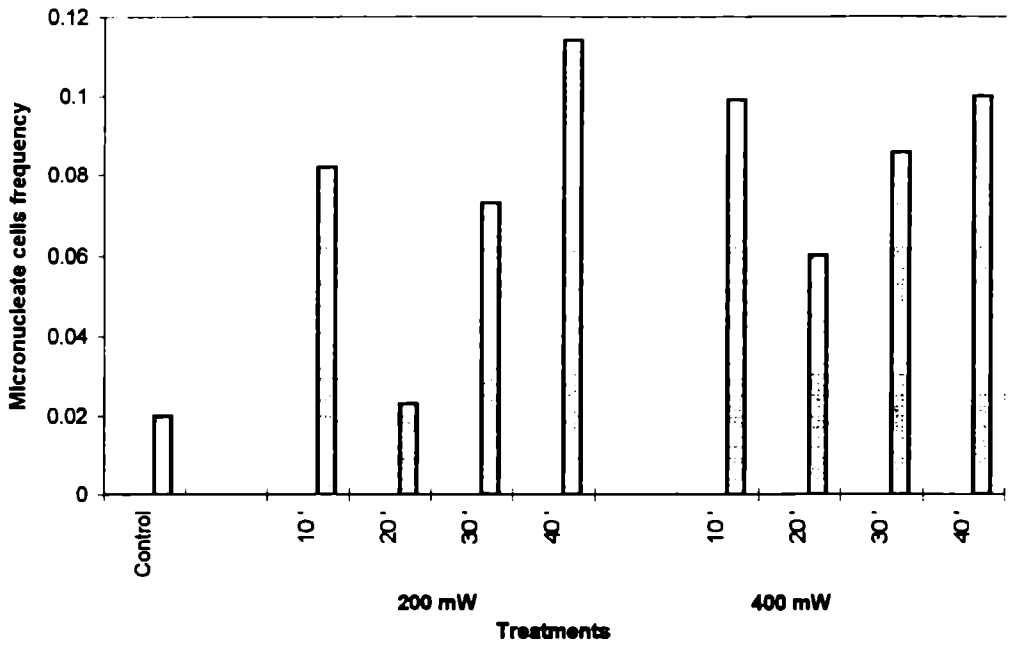


Fig. 37B : Effect of laser exposure at 488 nm on micronucleate cells in *V. faba*

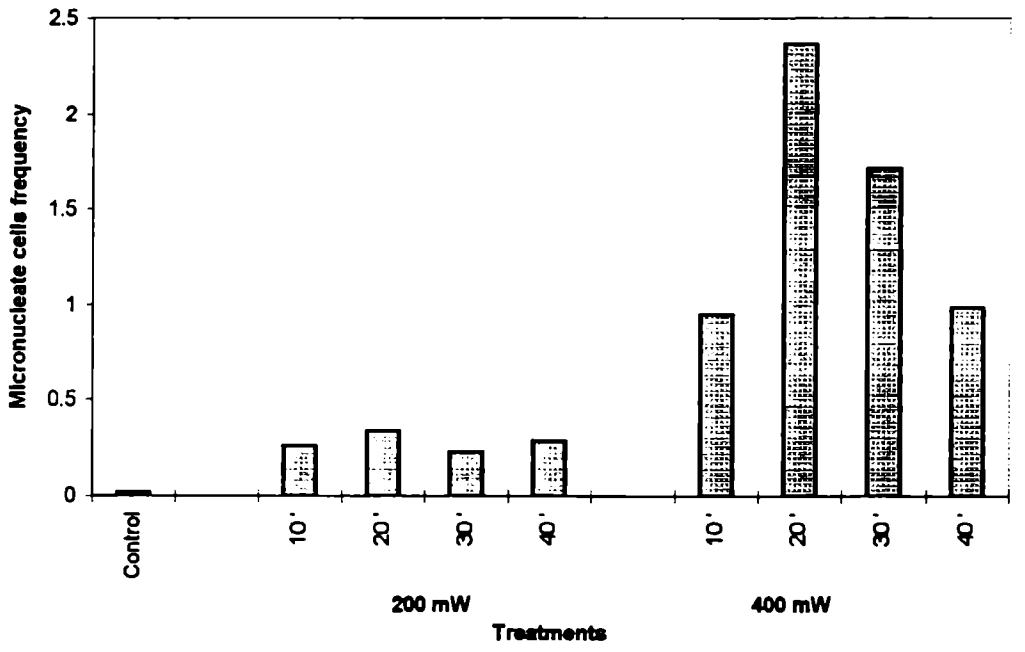


Fig. 38 B : Effect of laser irradiation at 514 nm on micronucleate cells in *V. faba*

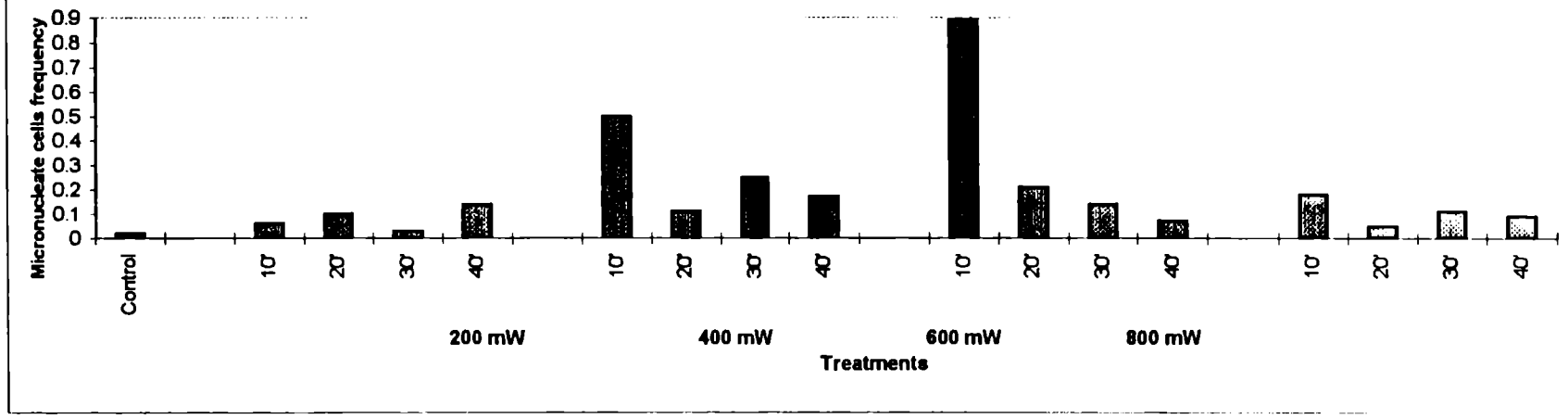


Fig. 39 B : Effect of UV exposure on micronucleate cells in *V. faba*

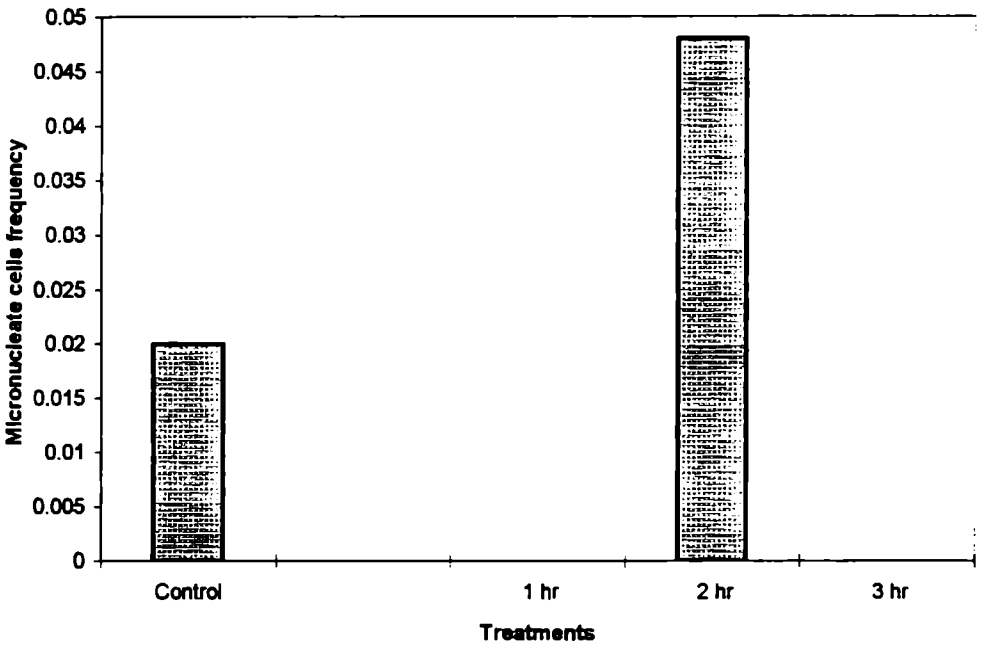


Fig. 40 B : Effect of gamma irradiation on micronucleate cells in *V. faba*

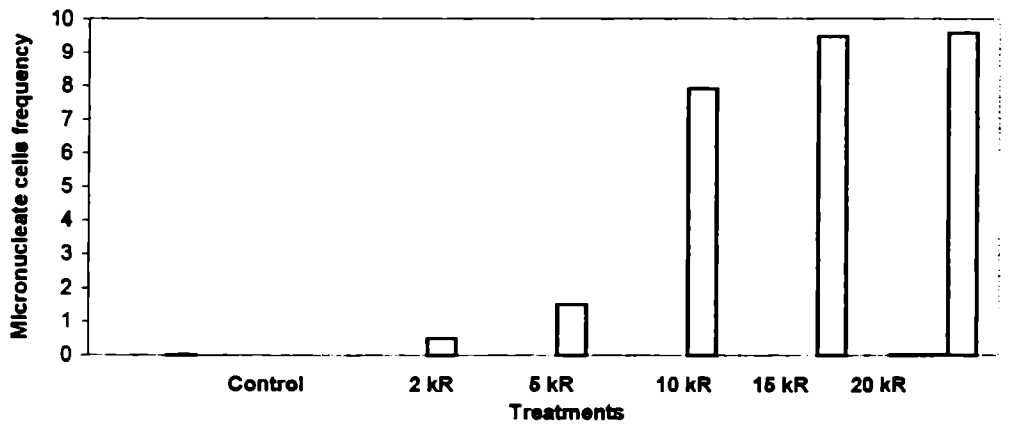


Fig. 41 B : Effect of EMS treatment on micronucleate cells in *V. faba*

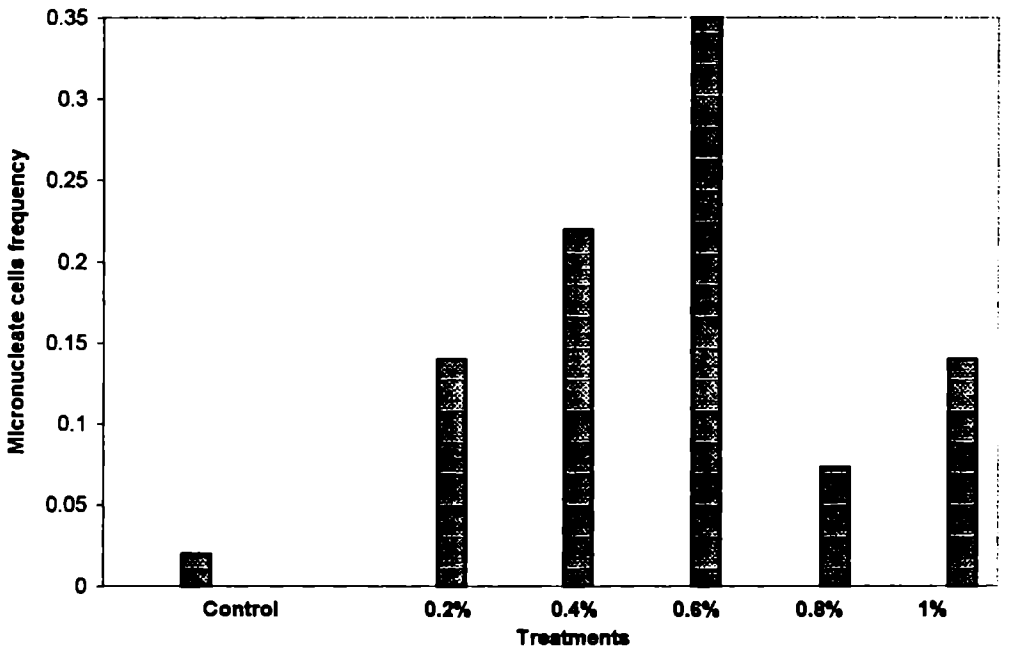
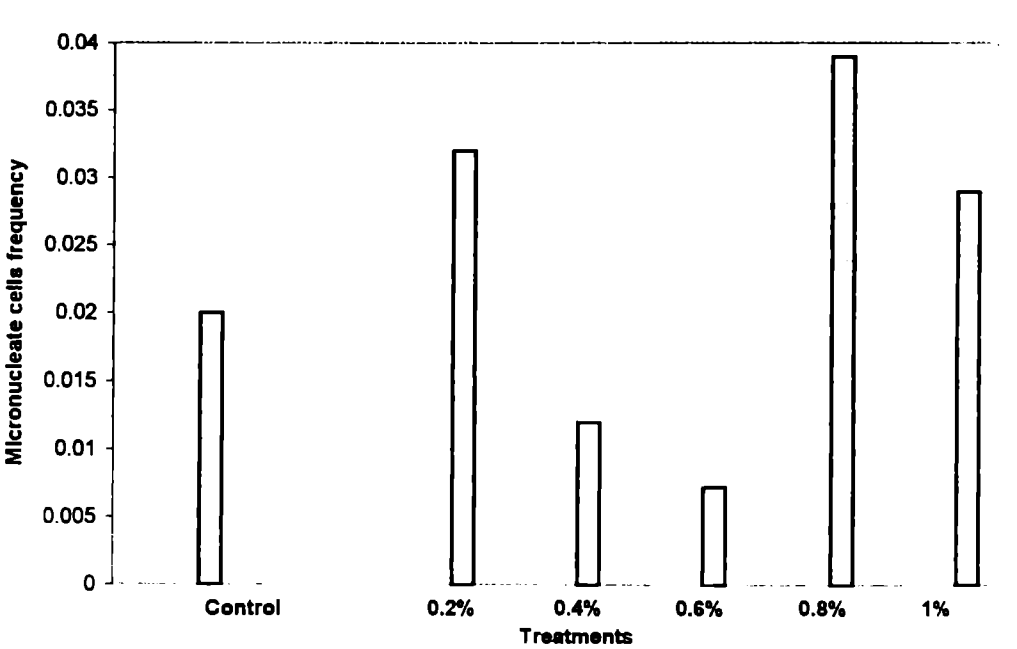


Fig. 42 B : Effect of HA treatment on micronucleate cells in *V. faba*



several fold higher than that induced by 476 nm and similar to 488 and 514 nm of laser exposures.

In *A. cepa,* HA treatment did not cause micronucleate cell formation (Fig.36A App.VIA). In *V. faba* HA treatment caused formation of micronucleate cells in low frequency (Fig.42B App.VIIB) The frequency was not dose dependent and was similar to that induced by 476 and several fold lower than that caused by 488 and 514 nm of laser exposures.

Micronucleate cells were observed in low frequency in some laser treatments in *A. cepa*, but in *V. faba* although seen in very low frequency at 476 it was observed in high frequencies at 488 and 514 nm laser exposures. Micronculeate cells were present in very low frequency in some UV exposures in both the species. EMS treatment induced micronucleate cells only in very low frequency in *A. cepa* but in *V. faba* it was induced in high frequency. No micronucleate cells were observed in HA treatment in *A. cepa* and it was present in very low frequency in *V. faba*.

4.2.4.2 Binucleate cells

Another type of aberration commonly found in most of the cases at telophase was the formation of binucleate cells. (plate figs.36, 61, 76, 116). It was seen in low frequency in the untreated controls of both plant species.

In *A. cepa,* 476 nm laser exposure caused binucleate cells in very low frequency in most doses but in high frequency on exposure for 40 min at the power density of 400 mW (Fig.37A App.IA). At 488 nm also this parameter was induced in low frequency. Although absent in exposure for 10 min it was present in high frequency in exposures of 40 min in the power

density of (Fig.38A App.IIIA). The frequency was not dose dependent. Laser exposures of 488 nm and 514 nm laser exposure induced dose dependent increase in binucleate cells in very high frequency with increase in treatment dose (Fig.39A App.IIIA). Binucleate cells were several fold more frequent in exposures of 514 nm than of 476 and 488 nm. In *V. faba* lasers of 476 and 488 nm induced dose dependent increase in binucleate cells frequency with increase in treatment dose (Figs.43B and 44B App.IB and IIB). No dose dependence on the induction of binculate cells by different doses of lasers of 514 nm was noticed but the frequency of this change was several fold higher in samples exposed for 10 min at the power density 600 mW than that of other treatment doses (Fig.45B App.IIIB) The frequency of binucleate cells induced by 476 and 488 nm lasers were higher than that induced by 514 nm.

In *A. cepa,* UV exposure caused dose dependent decrease in binuclate cell frequency with increase in exposure time (Fig.40A App.IVA). The frequency of induction of binucleate cells was similar to that induced by 476 and 488 nm lasers and several fold lower to that induced by 514 nm of laser exposure. In *V. faba*, UV exposure induced no dose dependent change in binclueate cell frequency (Fig.46B App.IVB). The frequency of this change was several fold lower than that induced by laser exposures.

γ-irradiations in *V. faba* induced dose dependent decrease in binculate cell frequency with increase in irradiation dose (Fig.47B App.VB). The frequency was lower than that of 476 and 488 nm and similar to 514 nm of laser exposures.

In *A. cepa* EMS treatment caused dose dependent increase the frequency of the formation of binculate cells with increase in treatment concentration (Fig.41A App.VA). The frequency was higher than that of 476 and 488 nm and several fold lower than 514 nm laser exposures. In *V. faba* also, dose dependent increase in the frequency of this change was induced by EMS treatment (Fig.48B App.VIB). The frequency was similar to 514 nm but lower than 476 and 488 nm laser exposure.

HA treatment in *A. cepa* induced dose dependent increase in binuclate cell frequency with increase in treatment dose (Fig.42A App.VIA). The frequency was higher than that of 476 and 488 nm and several fold lower than 514 nm laser exposure. In *V. faba*, the frequency of binculate cells showed no dose dependence and was several fold lower than the frequency of this change induced by laser exposures (Fig.49B App.VIIB).

Comparing the binucleate cell frequency in *A. cepa* and *V. faba*, laser exposures of 476 and 488 nm induced dose dependent decrease but 514 nm induced dose dependent increase in binculate cell frequency in *A. cepa* while in *V. faba* 476 nm and 488 nm laser exposures induced dose dependent increase in binucleate cell frequency but no dose dependence in 514 nm of laser exposures. UV caused dose dependent decrease in *A. cepa* and no dose dependent change in *V. faba* in binucleate cell frequency. EMS treatment caused dose dependent increase in binucleate cell frequency in both plant species. HA treatment caused dose dependent increase in *A. cepa* and no dose dependence in *V. faba* in binculeate cell frequency.

Fig. 37 A : Effect of laser exposure at 476 nm on binucleate cells in *A. cepa*

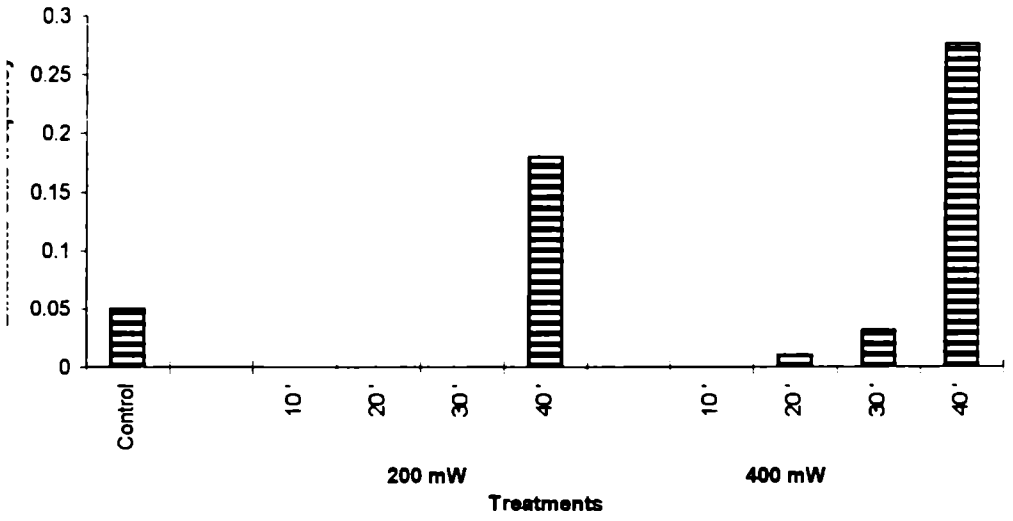


Fig. 38 A : Effect of laser exposure at 488 nm on binucleate cells in *A. cepa*

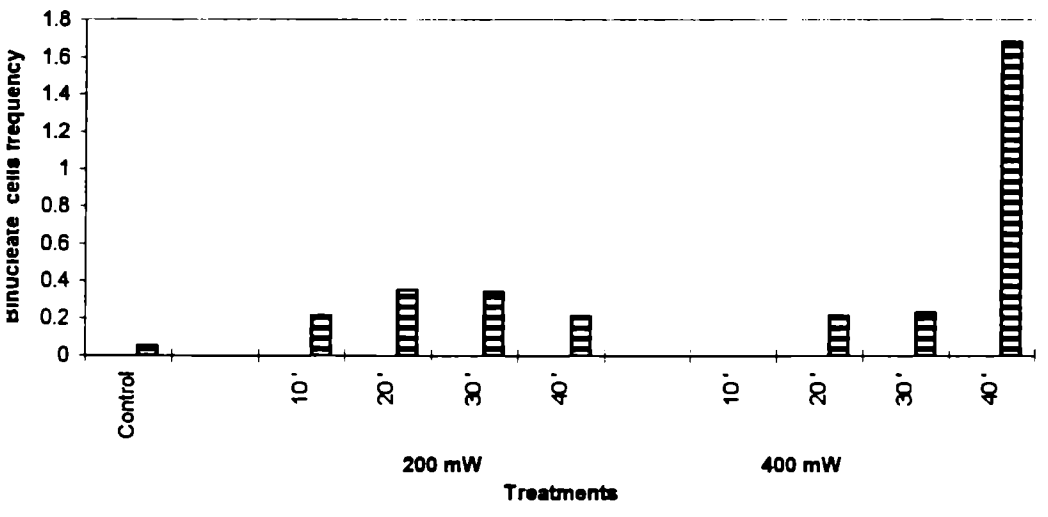


Fig. 39 A : Effect of laser irradiation at 514 nm on binucleate cells in *A. cepa*

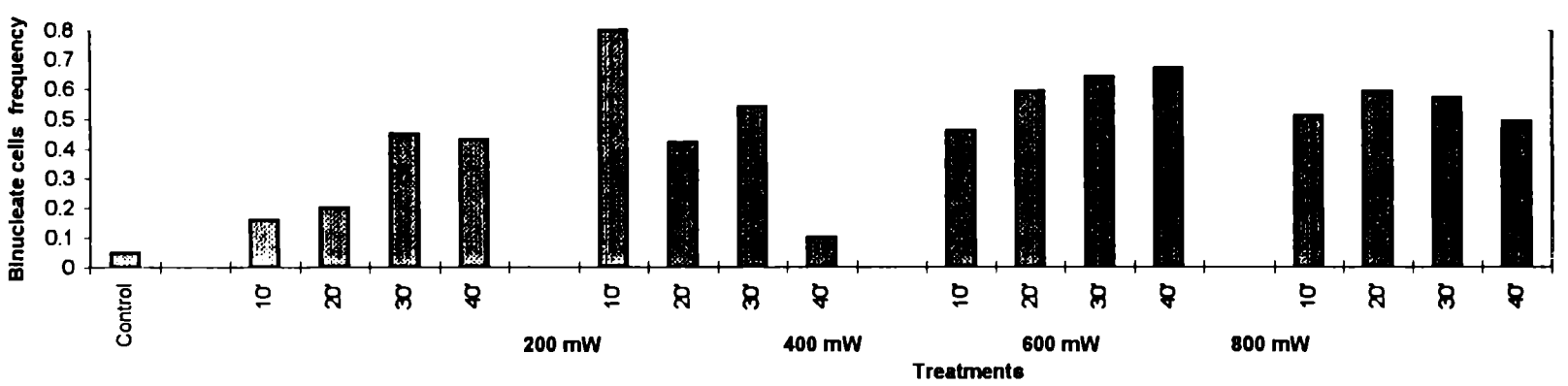


Fig. 40 A : Effect of UV exposure on binucleate cells in *A. cepa*

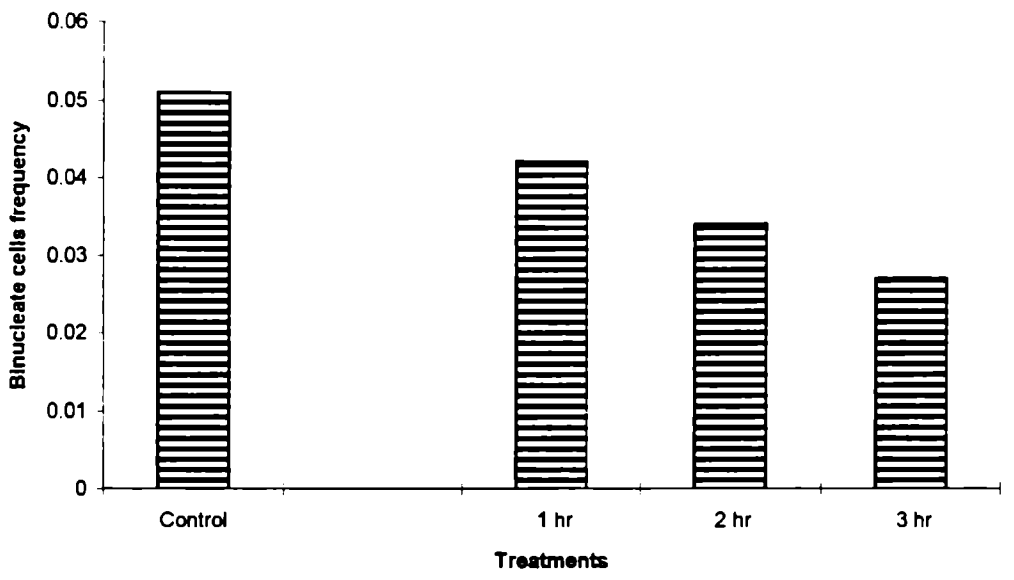


Fig. 41 A : Effect of EMS treatment on binucleate cells in *A. cepa*

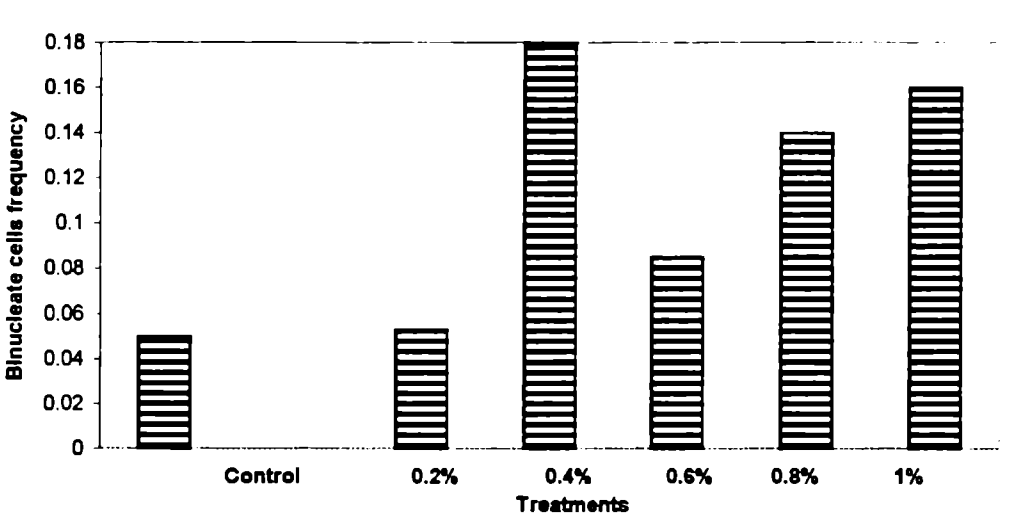


Fig. 42 A : Effect of HA treatments on binucleate cells in *A. cepa*

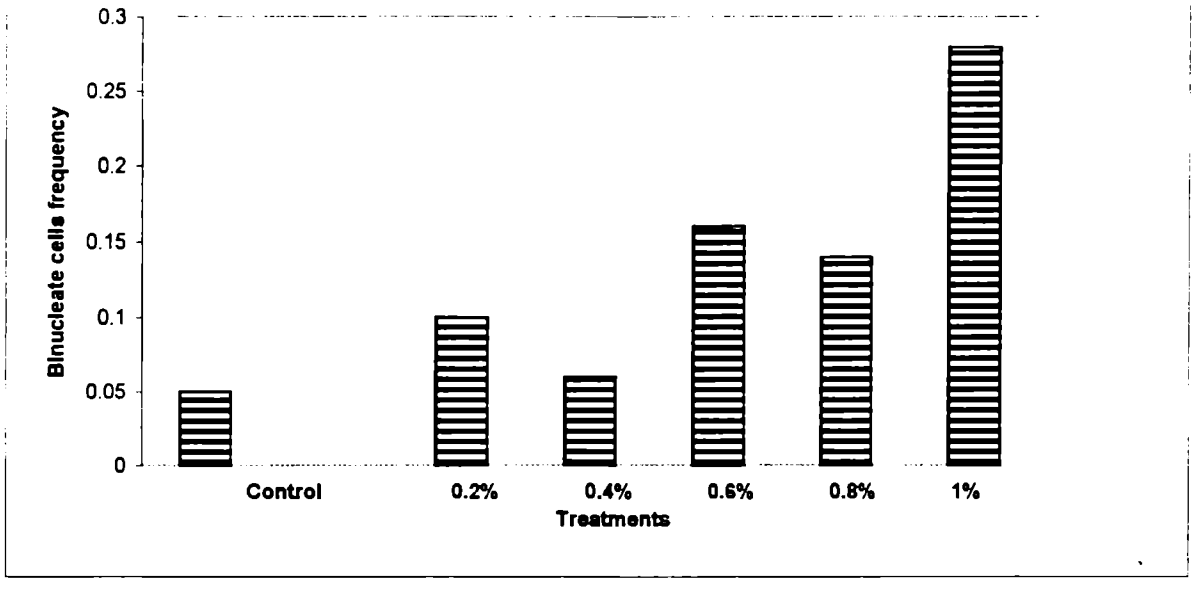


Fig. 43 B : Effect of laser exposure at 476 nm on binucleate cells in *V. faba*

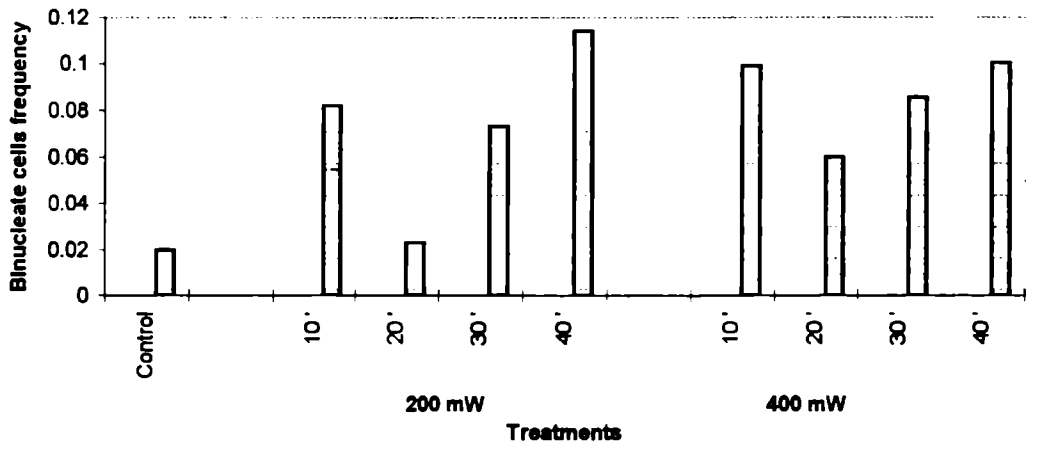


Fig. 44 B : Effect of laser exposure at 488 nm on binucleate cells in *V. faba*

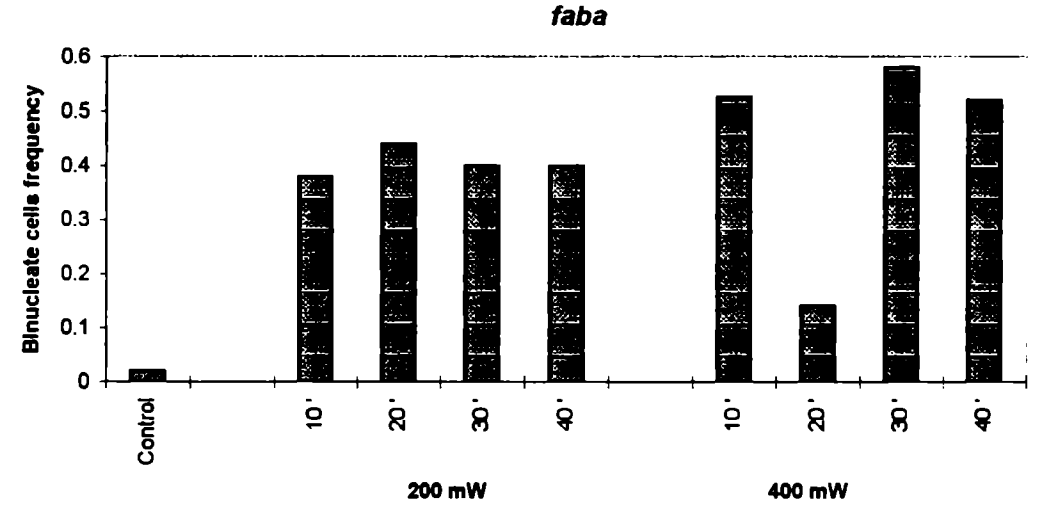


Fig. 45 B : Effect of laser irradiation at 514 nm on binucleate cells in *V. faba*

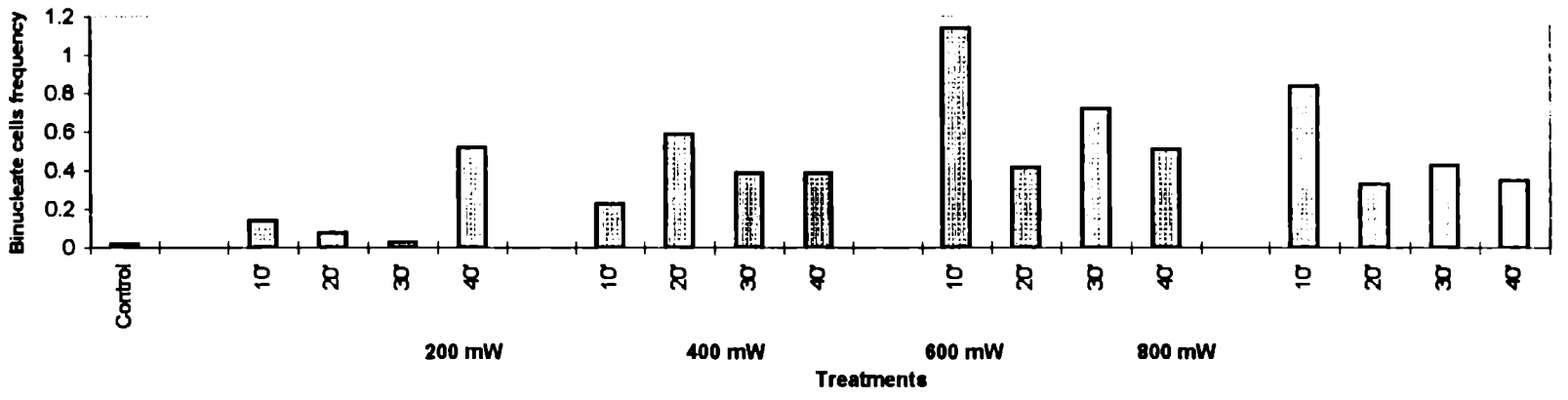


Fig. 46 B : Effect of UV exposure on binucleate cells in *V. faba*

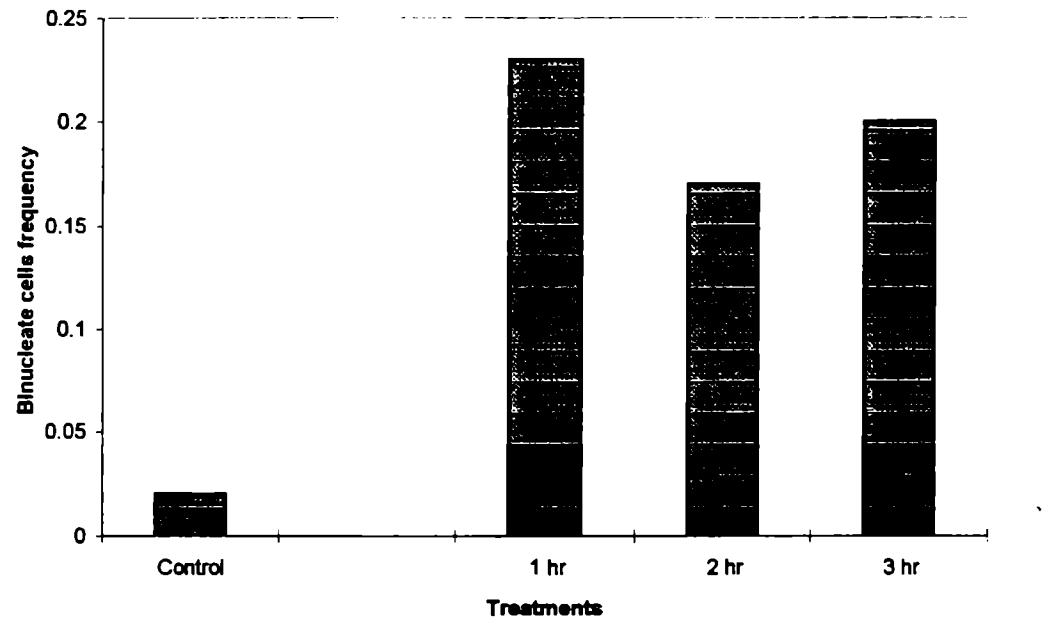


Fig. 47 B : Effect of gamma irradiation on binucleate cells in *V. faba*

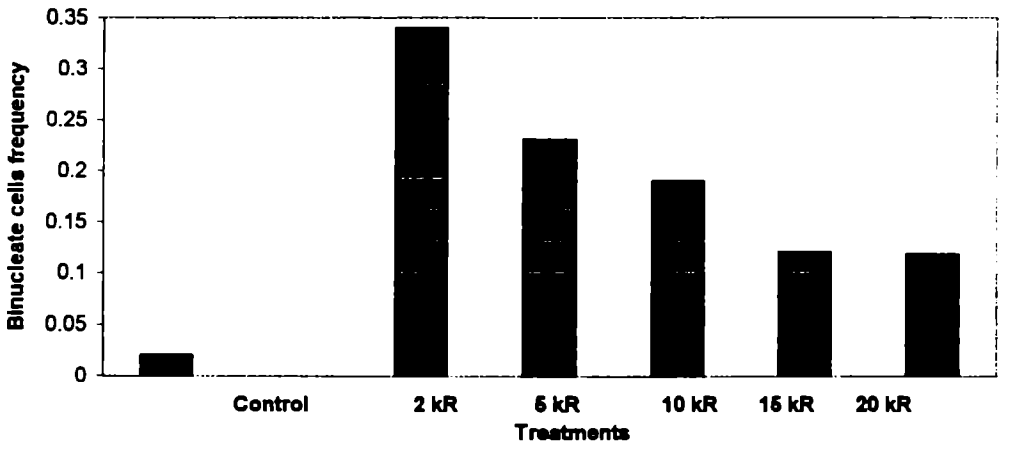


Fig. 48 B : Effect of EMS treatment on binucleate cells in *V. faba*

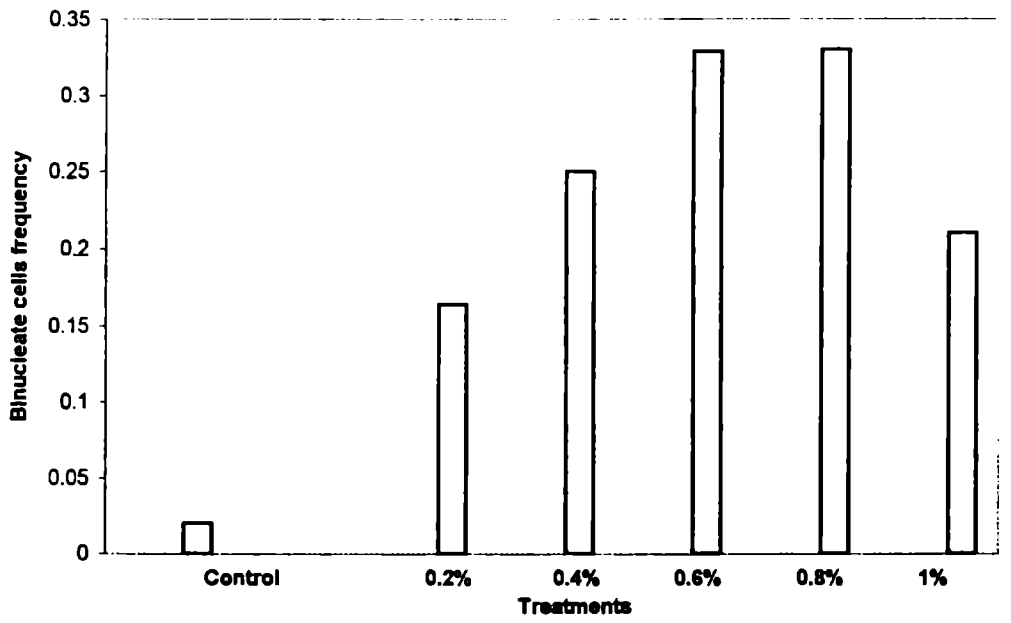
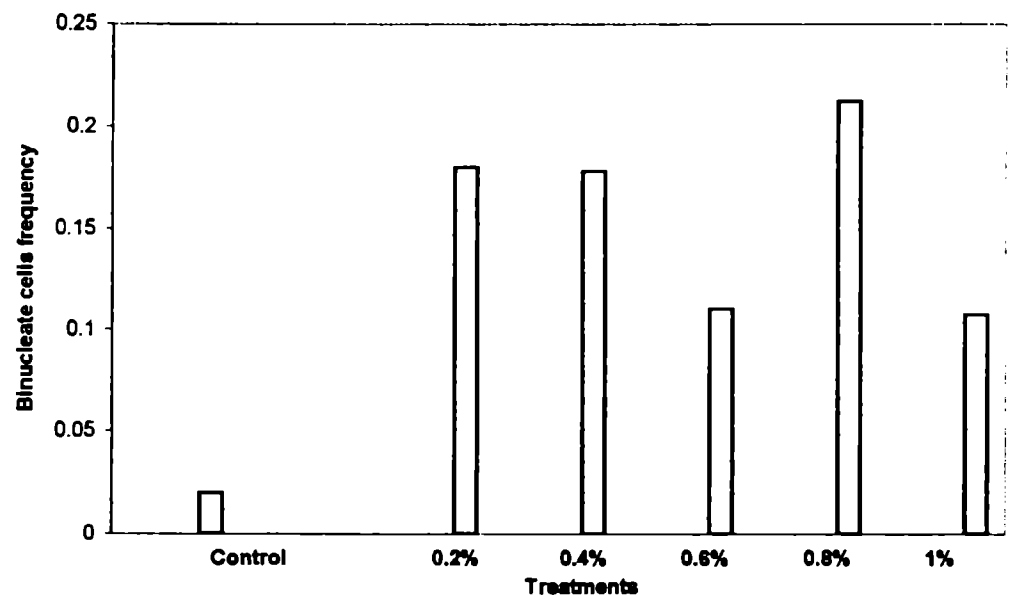


Fig. 49 B : Effect of HA treatment on binucleate cells in *V. faba*



4.2.4.3 Elongated nucleate cells

The presence of cells with highly elongated nuclei were another aberration seen at telophase (plate figs.7, 15, 37, 46, 47, 63). It was absent in controls of both plant species. In the case of treatments, this change was present in high frequency in most of the doses.

In *A. cepa,* laser exposures at 476 nm induced dose dependent increase in the frequency of the formation of elongated nuclei with increase in power density (Fig.43A App.IA) although at 488 nm no dose dependence was noticed in the frequency of this aberration (Fig.44A App.IIA). 514 nm laser exposure induced dose dependent decrease in the frequency of this change with increase in power density (Fig.45A App.IIIA). The elongated nucleate cell frequency at 476 and 488 nm were similar and 514 nm several fold lower than 476 and 488 nm. In *V. faba,* laser exposures of 476 nm caused dose dependent decrease in elongated cell nucleate frequency with increase in power density. The frequency of this change at 10 min of exposure to lasers of power density 200 mW was several fold lower than the same at other doses (Fig.50B App.IB). The frequency of this parameter at 488 nm showed dose dependent increase with increase in power density. However in exposures of 10 min at 400 mW the frequency of the change was very low (Fig.51B App.IIB). On exposure to lasers of 514 nm no dose dependence was observed but exposure for 20 min with lasers of power density 600 mW induced a frequency of change several fold higher to other doses (Fig.52B App.IIIB). The frequency of elongated nucleate cells at 476 nm was higher than that of 400 mW of 488 nm and several fold higher than that of 200 mW of 488 nm and 514 nm laser exposures.

In *A. cepa* on exposure to UV, induction of elongated nuclei showed no dose dependence (Fig.46A App.IVA). The frequency of elongated nucleate cells induced by UV was similar to that induced by 488 but was lower than the frequency of this change induced by 476 and higher than that induced by 514 nm laser exposures. In *V. faba* also, UV exposures did not cause dose dependent change in the frequency of formation of elongated nuclei (Fig.53B App.IVB). The frequency was similar to that induced by 488 nm, lower than that caused by 476 nm and several fold higher than that induced by 514 nm laser exposures.

γ-irradiations in *V. faba* induced no dose dependent change in elongated nucleate frequency (Fig.54B App.VB). The frequency of the change induced by γ- irradiations was similar to that induced by lasers at 476 and by lasers at 488 nm of power density 400 mW and several fold higher than that induced by lasers of 514 nm and 488 nm at power density 200 mW.

In *A. cepa,* EMS treatment induced dose dependent increase in elongated nucleate cell frequency with increase in treatment concentration (Fig.47A App.VA). The frequency of this parameter caused by EMS treatment was similar to that induced by laser exposures at 476 and 488 nm and several fold higher than 514 nm. In *V. faba* also, EMS caused dose dependent increase in the frequency of this change with increase in treatment concentration (Fig.55B App.VIB). The frequency of the formation of elongated nuclei was several fold higher to that induced by laser exposures at 514 nm and 200 mW of 488 nm, slightly higher than 400 mW of 488 nm and similar to 476 nm.

In *A. cepa*, HA treatment caused dose dependent decrease in elongated nucleate cell frequency with increase in treatment concentration

Fig. 43 A : Effect of laser exposure at 476 nm on elongated nucleate cells in *A. cepa*

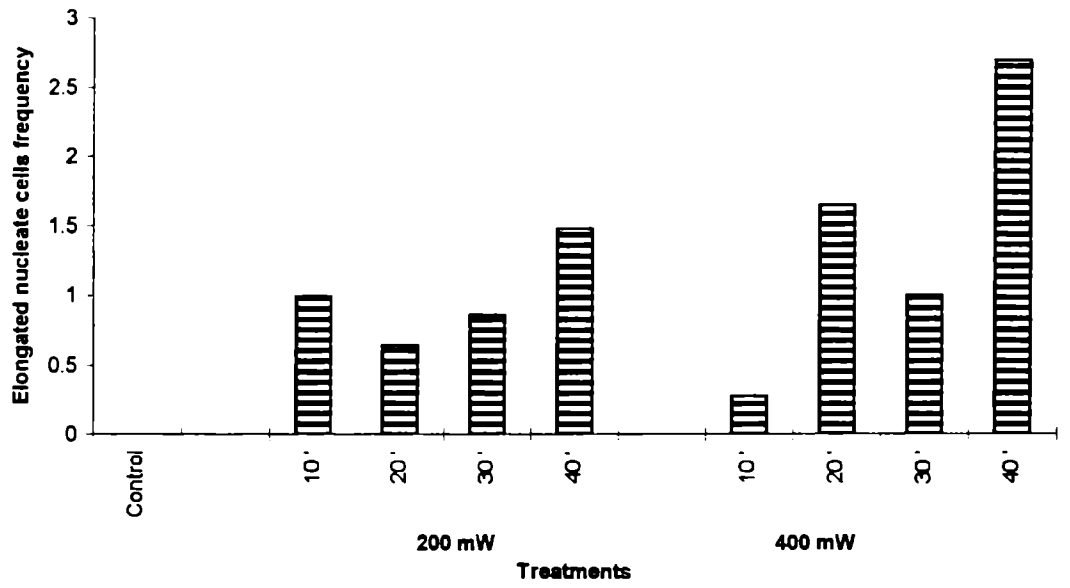


Fig. 44 A : Effect of laser exposure at488 nm on elongated nucleate cells in *A. cepa*

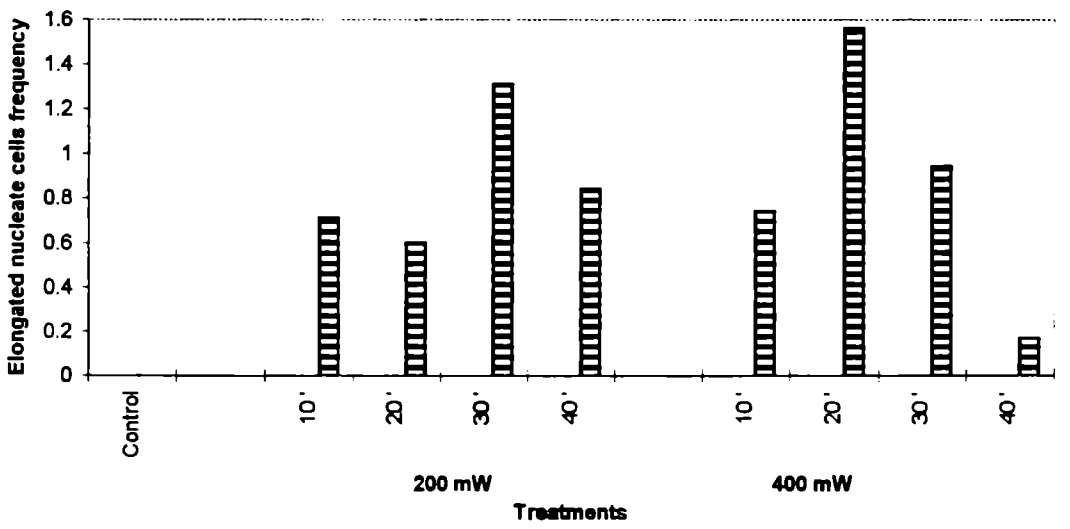


Fig. 45 A : Effect of laser irradiation at 514 nm on elongated nucleate cells in *A. cepa*

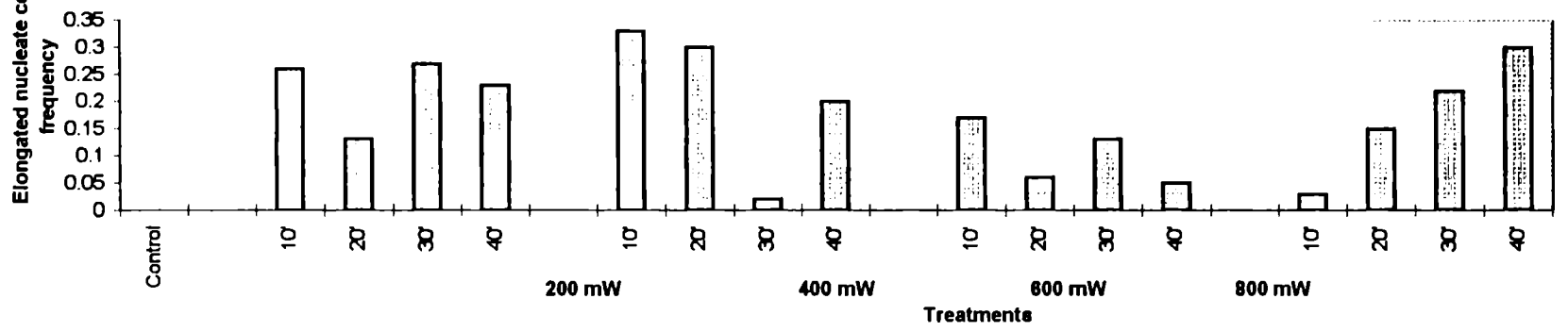


Fig. 46 A : Effect of UV exposure on elongated nucleate cells in *A. cepa*

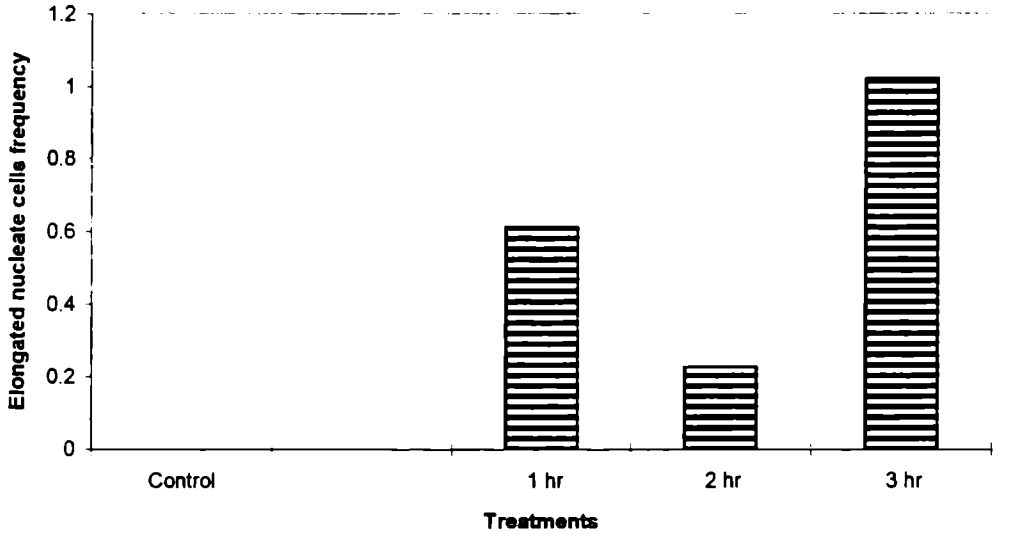


Fig. 47 A : Effect of EMS treatment on elongated nucleate cells in *A. cepa*

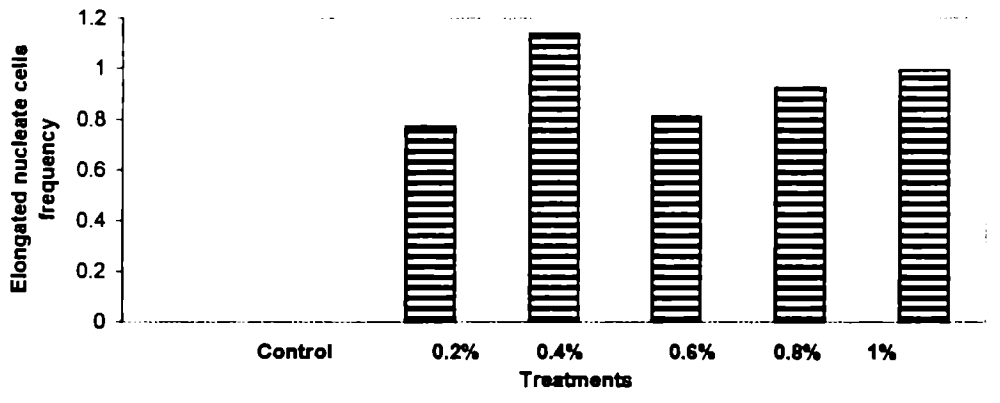


Fig. 48 A : Effect of HA treatment on elongated nucleate cells in *A. cepa*

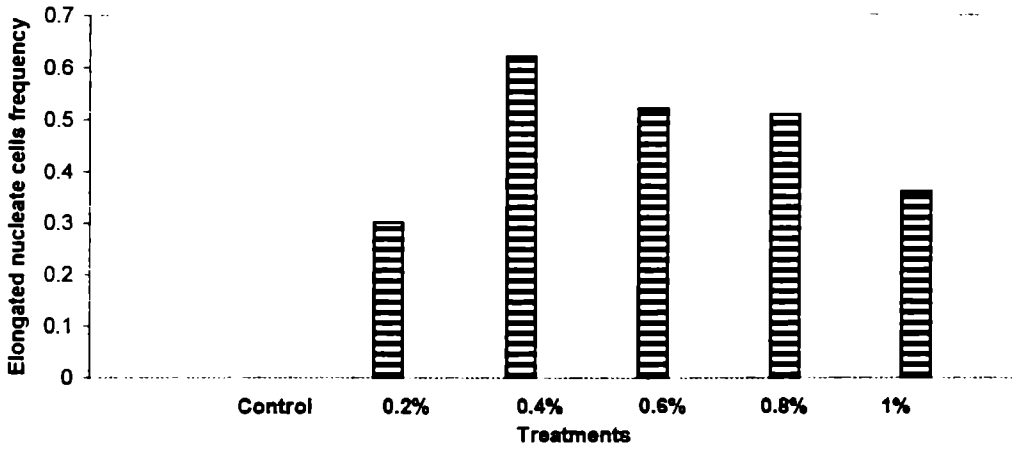


Fig. 50 B : Effect of laser exposure at 476 nm on elongated nucleate cells in *V. faba*

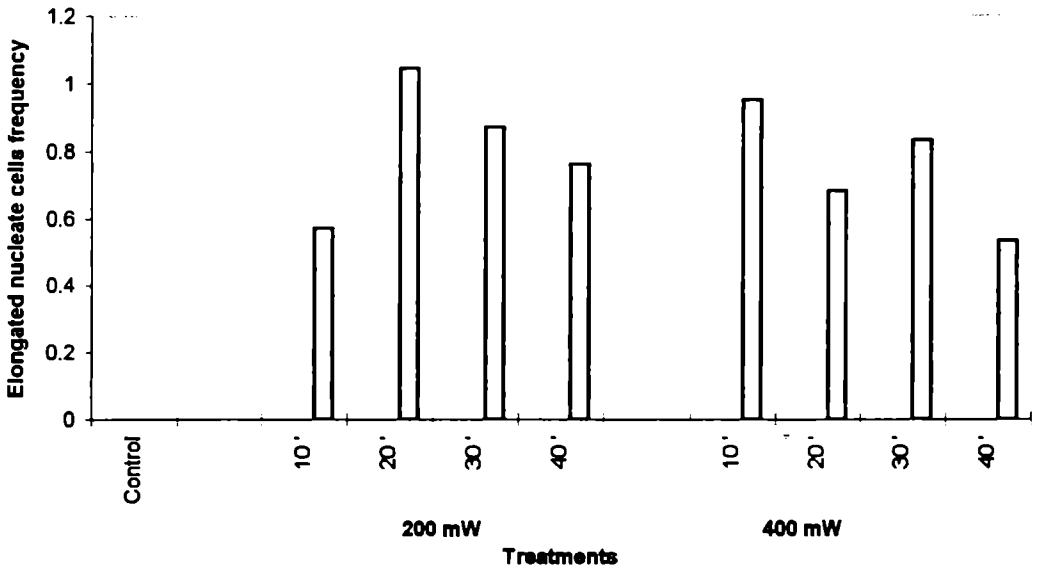


Fig. 51 B : Effect of laser exposure at 488 nm on elongated nucleate cells in *V. faba*

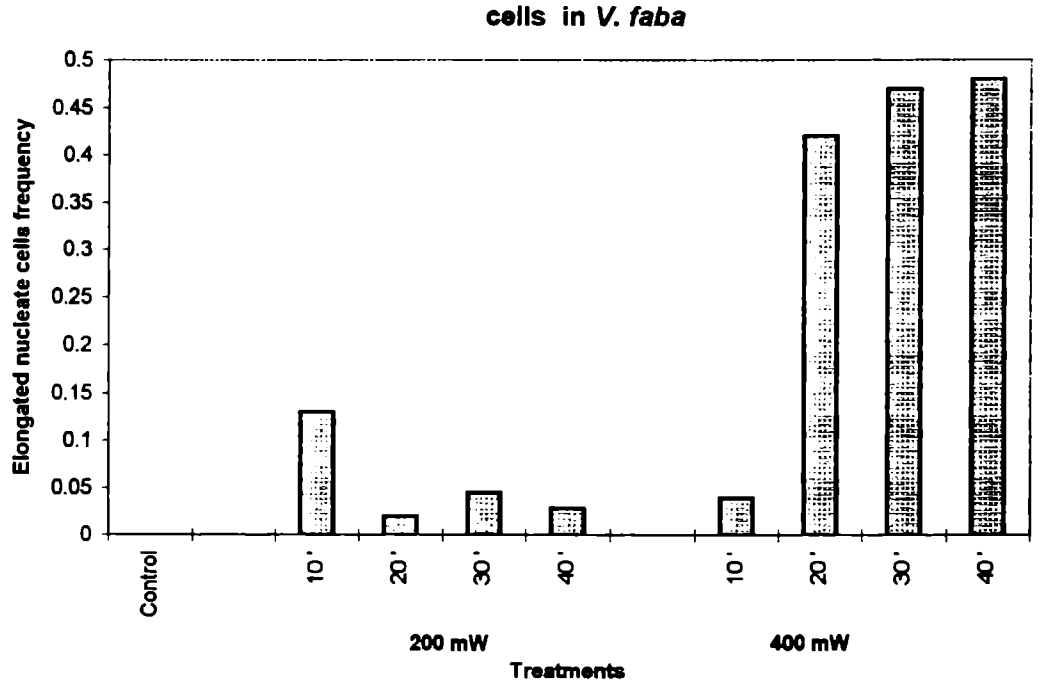


Fig. 52 B : Effect of laser irradiation at 514 nm on elongated nucleate cells in *V. faba*

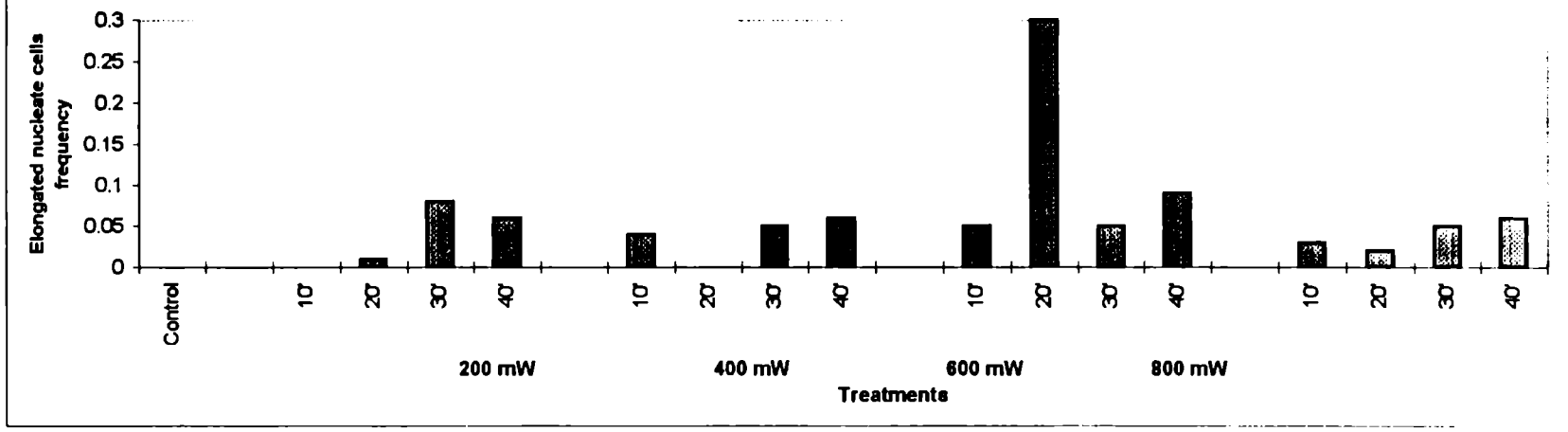


Fig. 53 B : Effect of UV exposure on elongated nucleate cells in *V. faba*

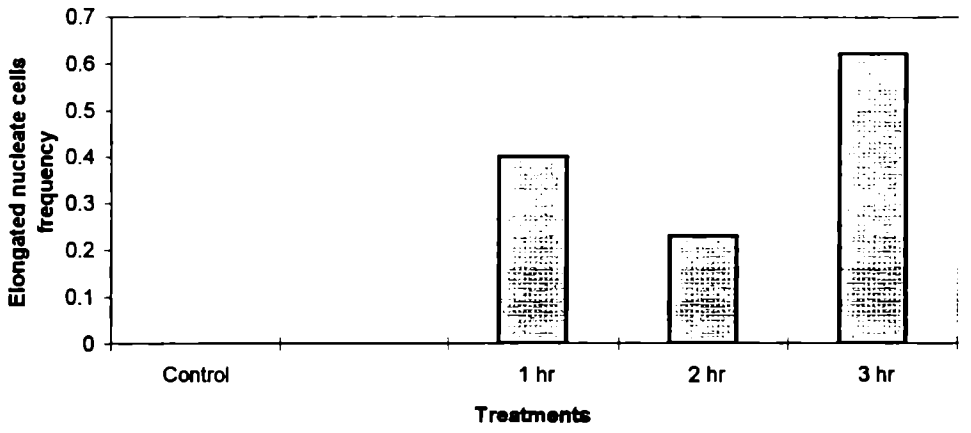


Fig. 54 B : Effect of gamma irradiation on elongated nucleate cells in *V. faba*

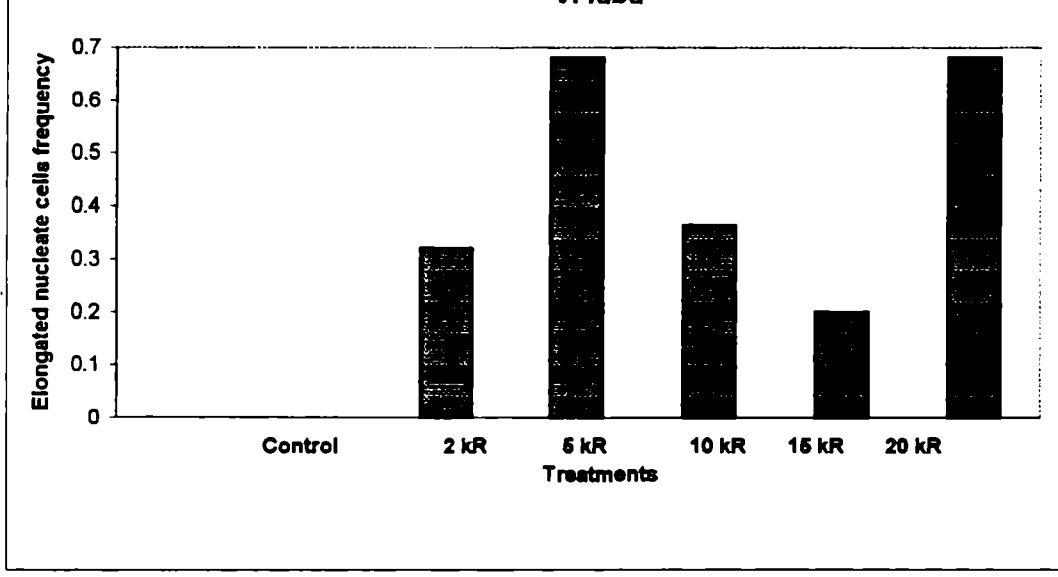


Fig. 55 B : Effect of EMS treatment on elongated nucleate cells in *V. faba*

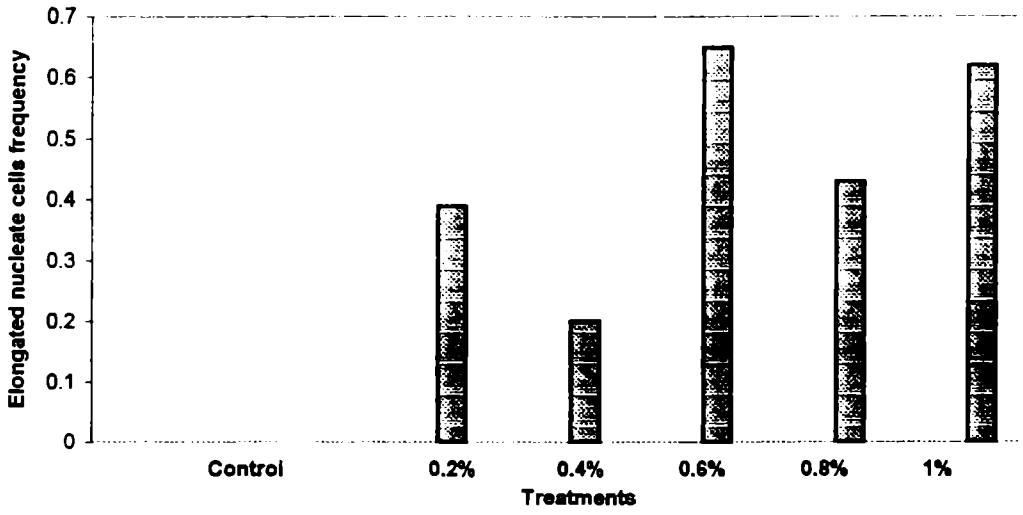
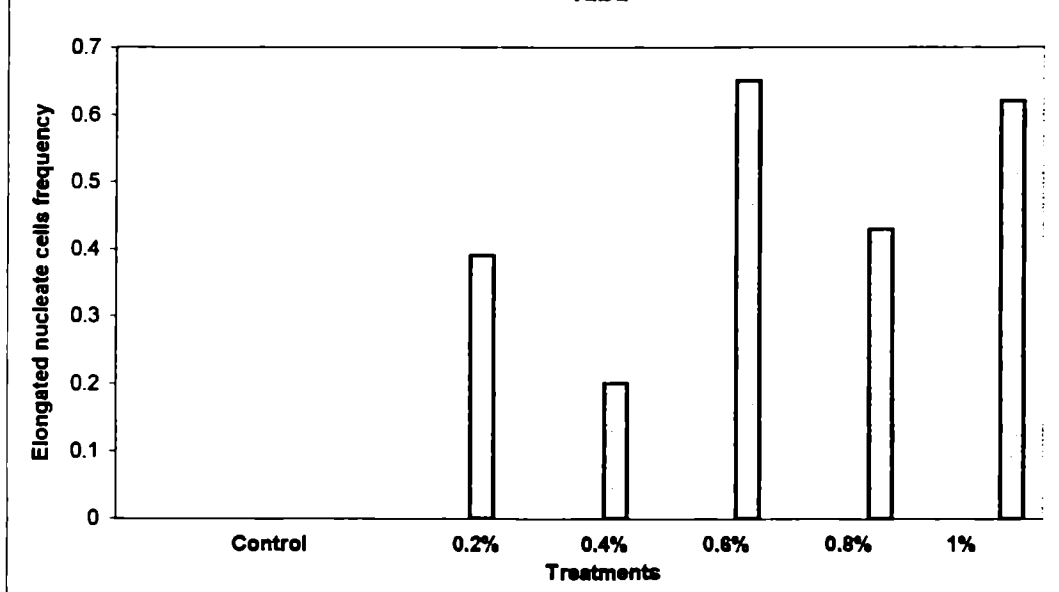


Fig. 56 B : Effect of HA treatment on elongated nucleate cells in *V. faba*



(Fig.48A App.VIA). The frequency of this parameter was higher than that induced by laser exposure at 514 nm and lower than 476 and 488 nm. In *V. faba*, HA treatment did not cause dose dependence in elongated nucleate cell frequency (Fig.56B App.VIIB). The frequency of this change caused by HA was higher to that induced by laser exposures at 514 nm and at 488 nm at power density 200 mW, but similar to 400 mW of 488 nm and lower than that induced by lasers of 476 nm.

On comparing between *A. cepa* and *V. faba* in the formation of elongated nucleate cell frequency, it is seen that laser exposures at 476 nm induced dose dependent increase in *A. cepa* but decrease in *V. faba*. Lasers of 488 nm caused dose dependent increase in the frequency of elongated nucleate cells in *V. faba* but not in *A. cepa*. 514 nm on the other hand caused a dose dependent decrease in *A. cepa* but not in *V. faba*. UV exposures induced no dose dependent changes in elongated nucleate cell frequency in both plant species. EMS treatments caused dose dependent increase in elongated nucleate cell frequency in both plant species while HA caused dose dependent increase in *A. cepa* and no dose dependence in *V. faba*.

4.2.5 Percentage of total mitotic aberration

The sum total of the different types of aberration induced by the mutagen treatments in four stages of mitotic cell division was used to determine the percentage of aberration.

In *A. cepa*, laser exposure at 476 nm and 488 nm induced dose dependent increase in total aberration frequency with increase in power density (Figs.49A and 50A App.IA and IIA), but no such dose dependence was observed with lasers of 514 nm (Fig.51A App.IIIA). In *V. faba,* laser exposures at 476

and 514 nm induced no dose dependent change in the frequency of total mitotic aberration (Figs.57B and 59B App.IB and IIIB) while lasers at 488 nm induced a dose dependent increase in frequency with increase in power density (Fig.58B App.IIIB). The highest frequency of aberration was elicited by 10 min exposure to 514 nm lasers of power density 600 mW.

In *A. cepa,* UV exposure induced dose dependent increase in frequency of total mitotic aberration with increase in exposure time (Fig.52A App.IVA). The frequency of this change was lower than that induced by lasers of 488 nm and 476 nm at a power density of 400 mW, but similar to that induced by other laser exposures. UV exposures induced dose dependent decrease in total aberration frequency with increase in exposure time in *V. faba* (Fig.60B App.IVB). The frequency of this change was lower than that induced by lasers of 488 nm at power density 400 mW but similar to other laser exposures.

γ - irradiations in *V. faba* induced dose dependent increase in total aberration frequency with increase in irradiation dose (Fig.61B App.VB). The frequency of change at 2 kR was lower than that induced by lasers of 488 at power density 400 mW and similar to other laser exposures. 5 kR frequency of this parameter was similar to that induced by 400 mW of 488 nm and higher than other laser exposures. Other total aberration frequency of γ - irradiations were several fold higher than that induced by laser exposures.

In *A. cepa,* EMS caused dose dependent increase in total aberration frequency with increase in EMS concentrations (Fig.53A App.VA). The frequency of total aberration caused by EMS was similar to that induced by lasers of 488 and 400 mW of 476 nm and higher than other laser exposures. In

Fig. 49 A : Effect of laser exposure at 476 nm on total mitotic aberrations frequency in *A. cepa*

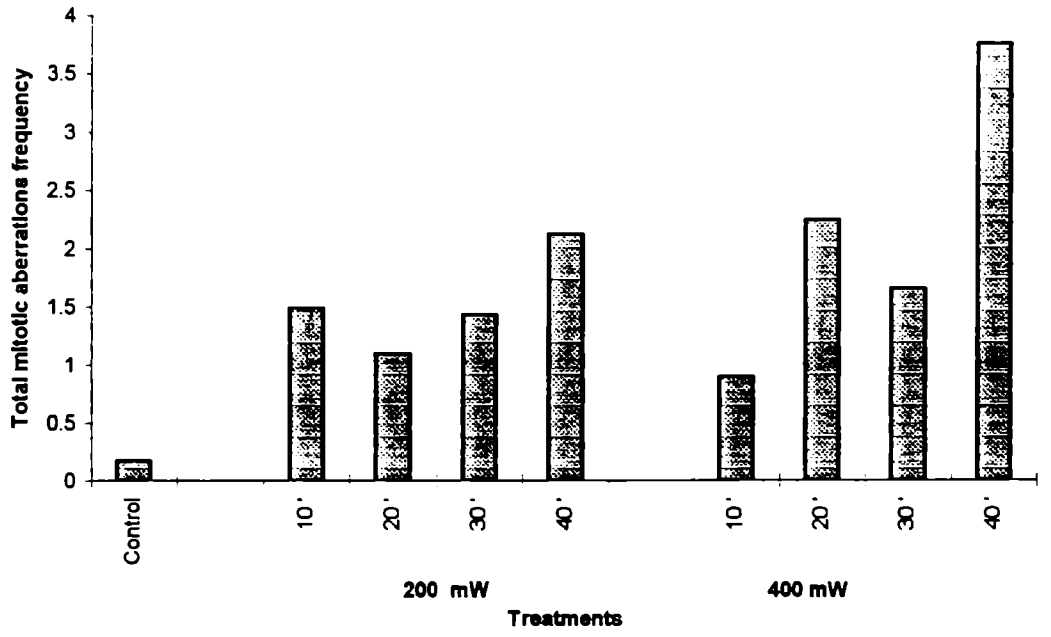


Fig. 50 A : Effect of laser exposure at 488 nm on total mitotic aberrations in *A. cepa*

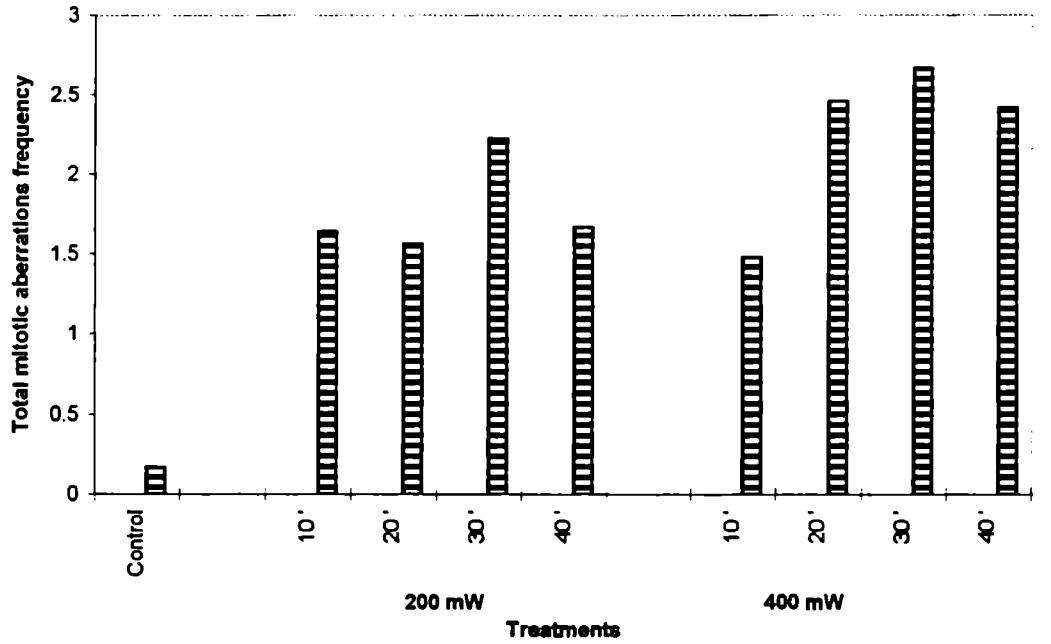


Fig. 51 A : Effect of laser irradiation at 514 nm on total mitotic aberrations frequency in *A. cepa*

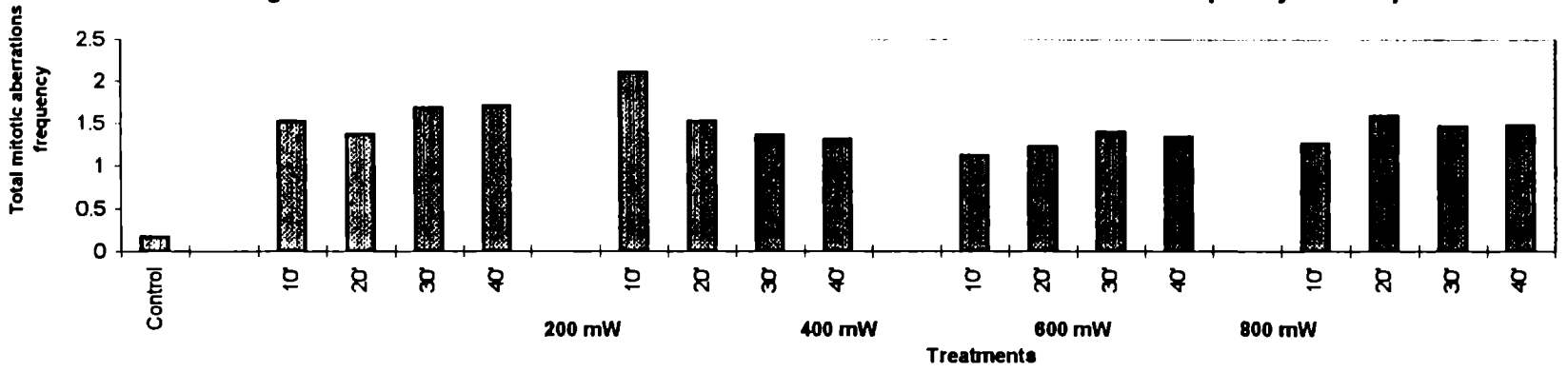


Fig. 52 A : Effect of UV exposure on total mitotic aberrations in *A. cepa*

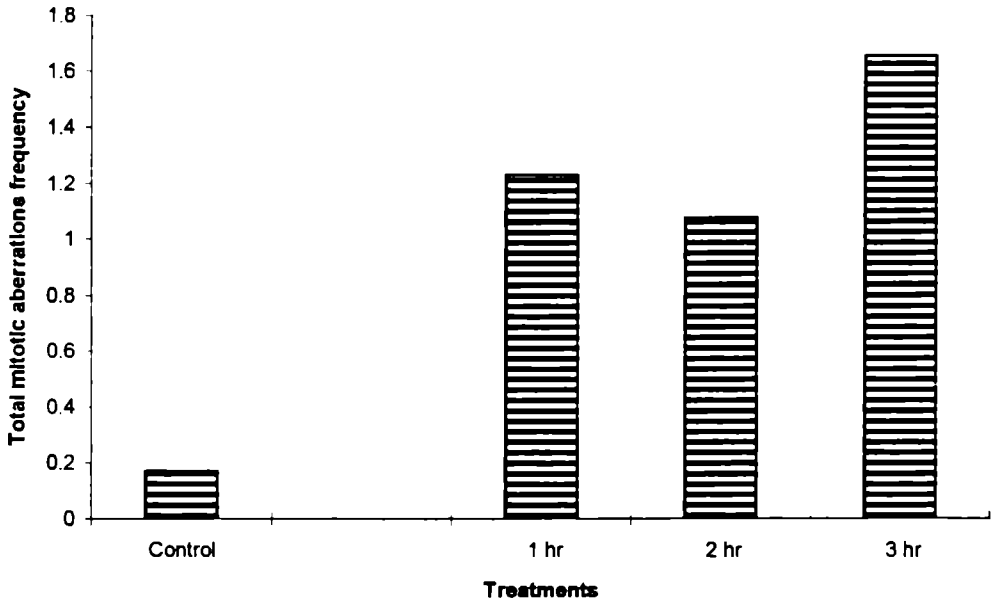


Fig. 53 A : Effect of EMS treatment on total mitotic aberrations in *A. cepa*

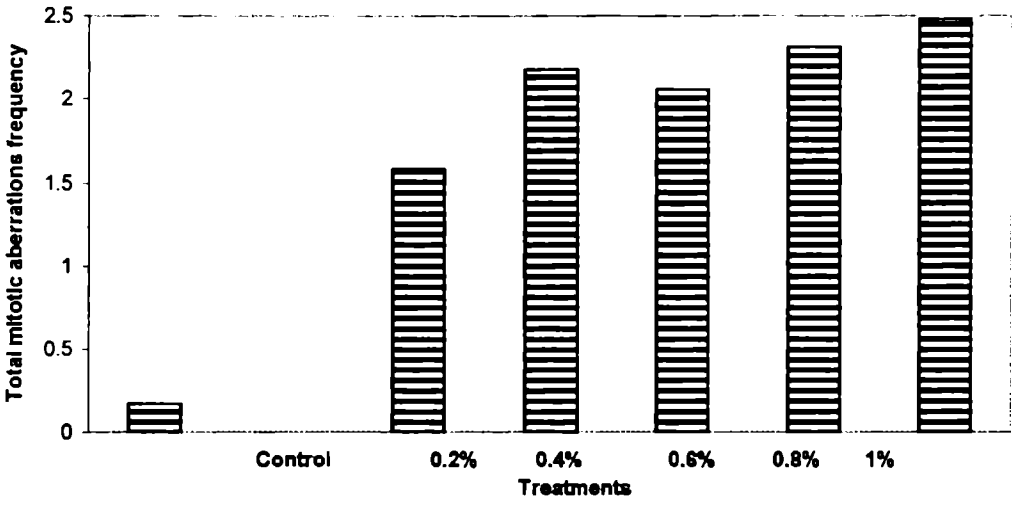


Fig. 54 A : Effect of HA treatment on total mitotic aberrations in *A. cepa*

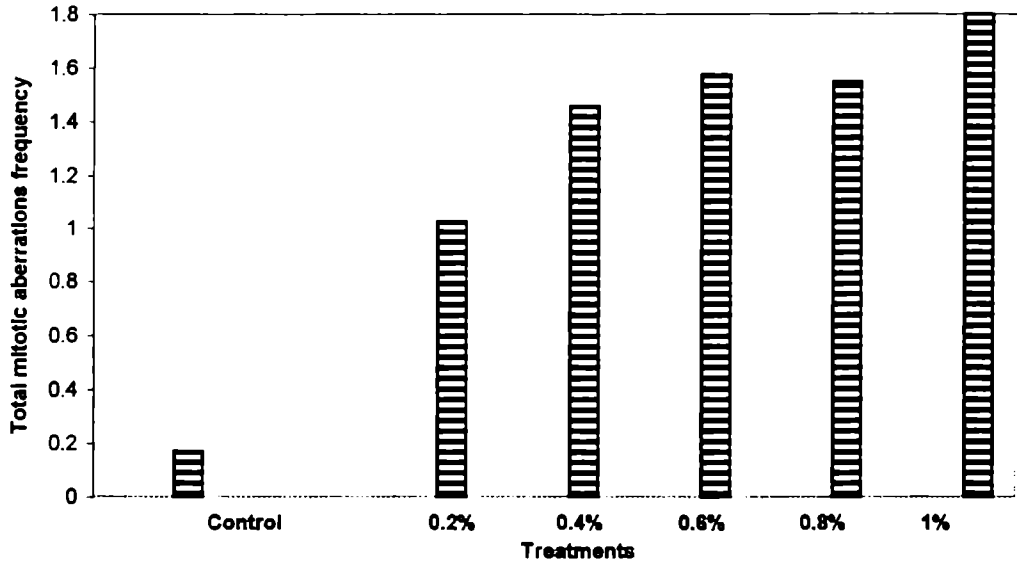


Fig. 57 B : Effect of laser exposure at 476 nm on total mitotic aberrations in *V. faba*

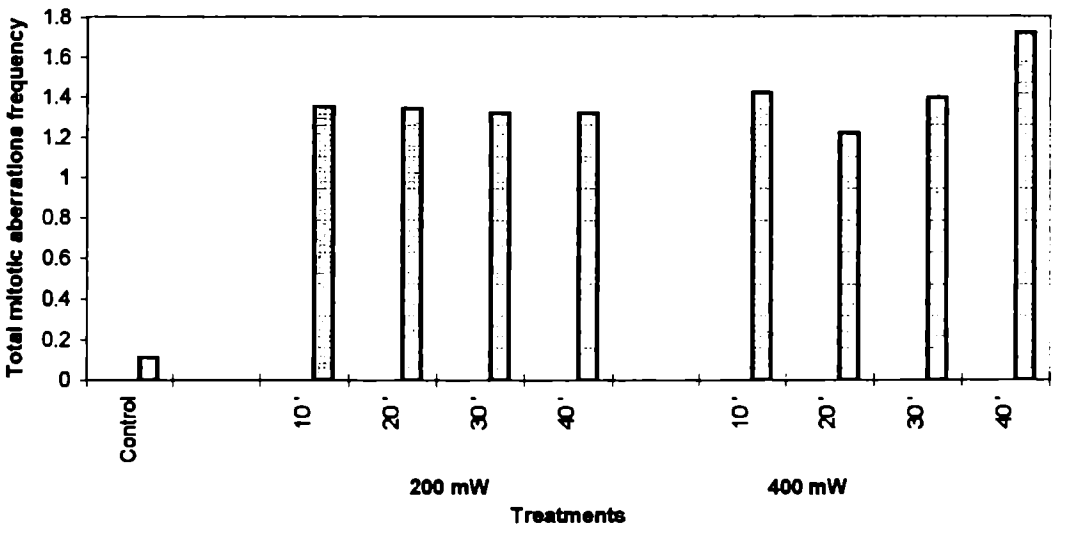


Fig. 58 B : Effect of laser exposure at 488 nm on total mitotic aberrations in *V. faba*

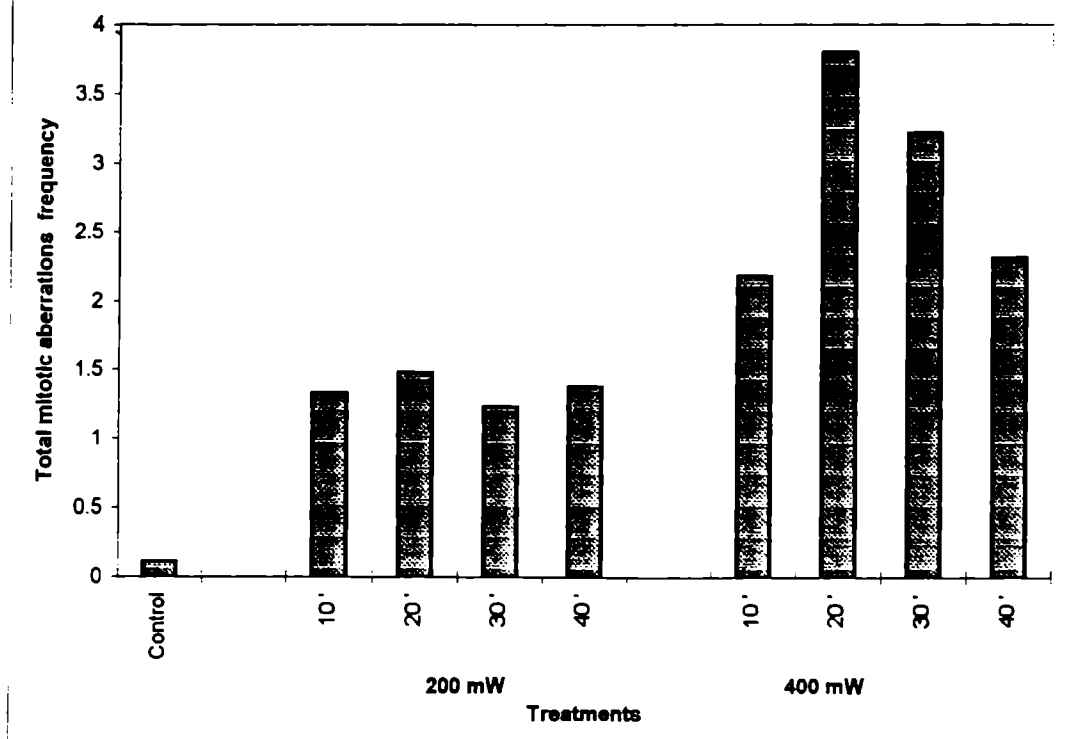


Fig. 59 B : Effect of laser irradiation at 514 nm on total mitotic aberrations in *V. faba*

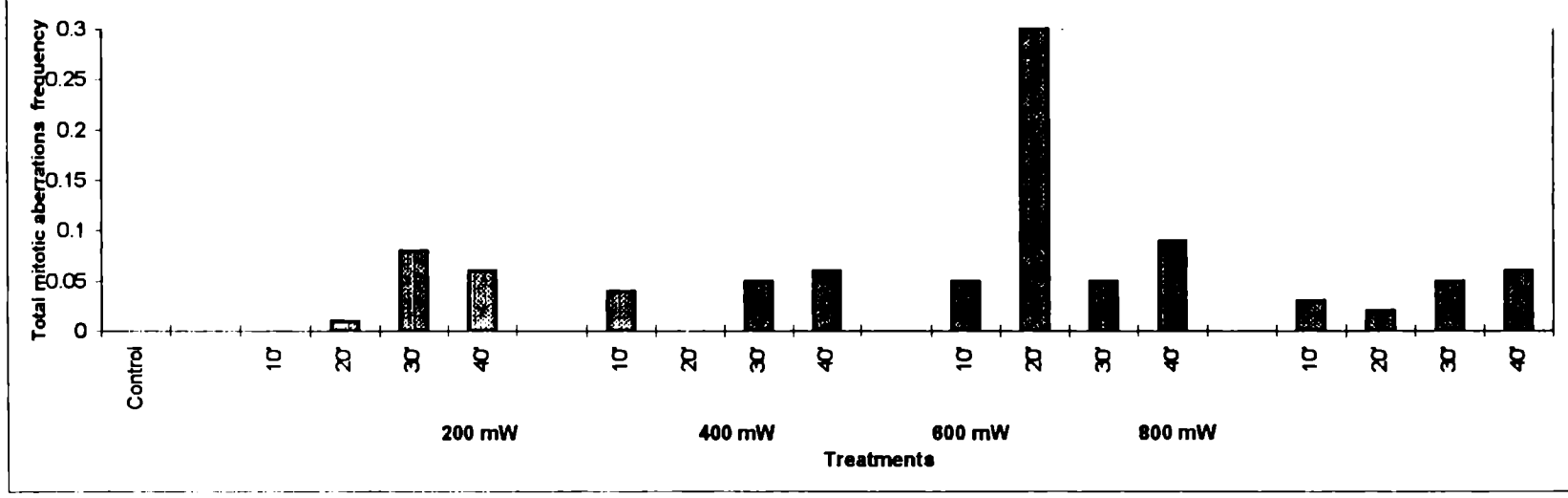


Fig. 60 B : Effect of UV exposure on total mitotic aberrations in *V. faba*

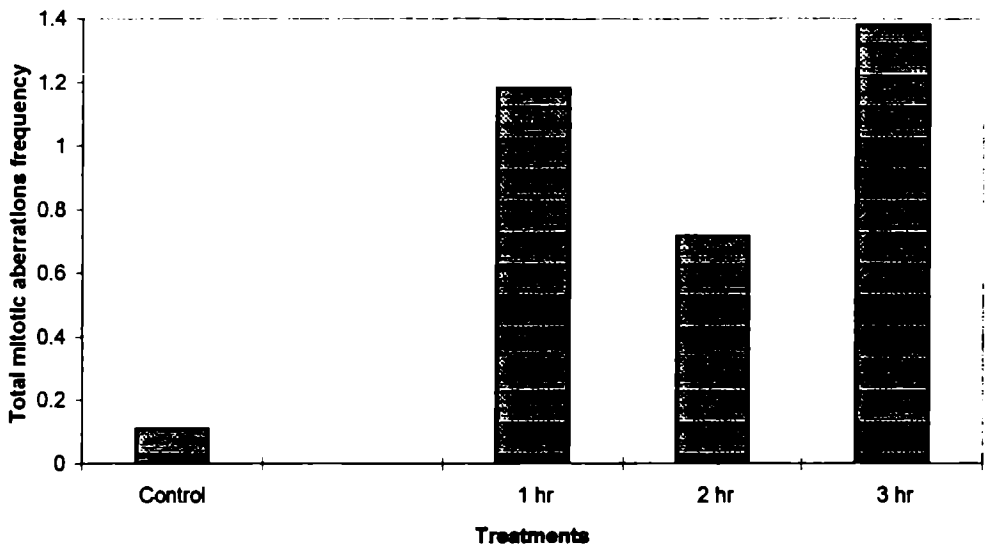


Fig. 61 B : Effect of gamma irradiation on total mitotic aberrations in *V. faba*

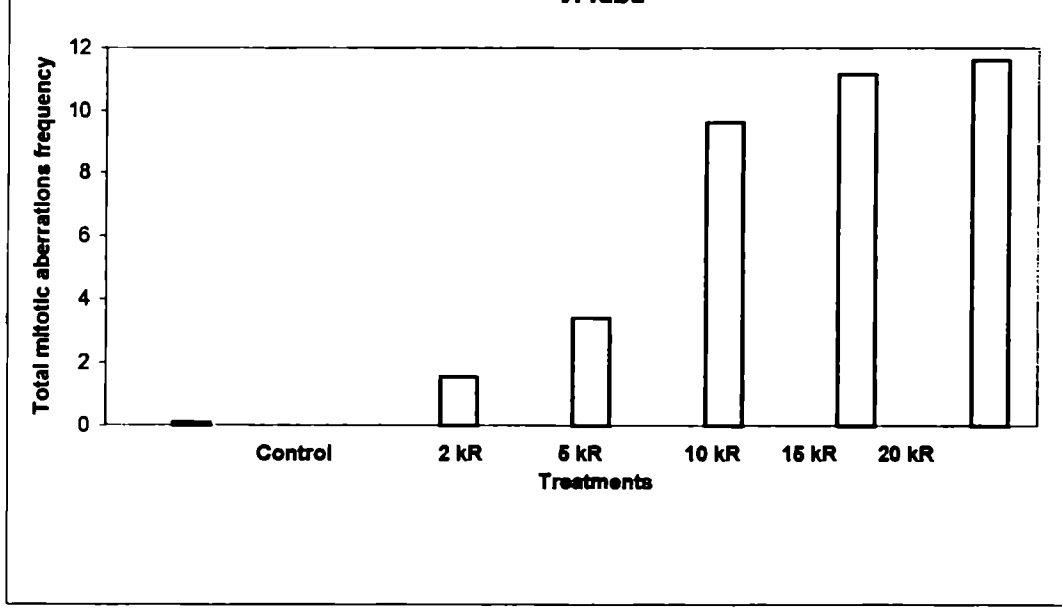


Fig. 62 B : Effect of EMS treatment on total mitotic aberrations in *V. faba*

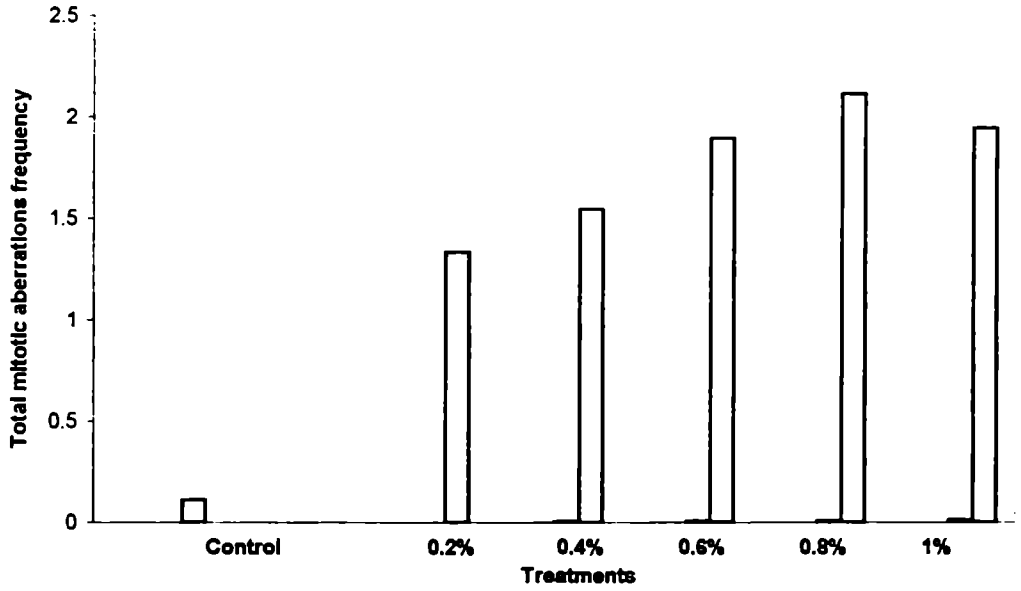
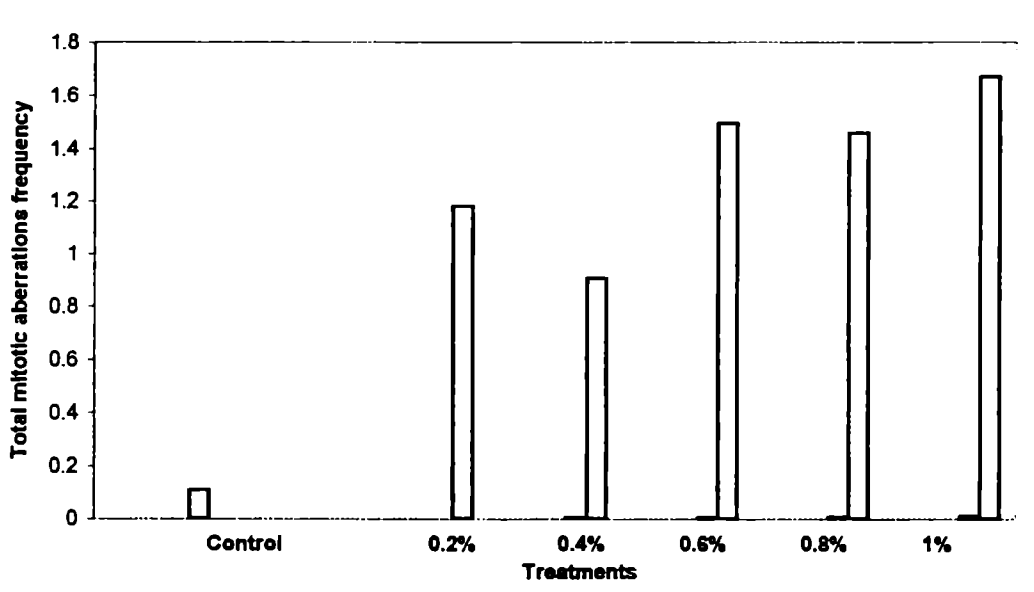


Fig.63 B : Effect of HA treatment on total mitotic aberrations in *V. faba*



V. faba also, EMS induced dose dependent increase in total aberration frequency with increase in concentration (Fig.62B App.VIB). Total aberration frequency in EMS was lower than that induced by laser exposure at 400 mW of 488 nm and similar to other laser exposures.

HA treatment in *A. cepa* induced dose dependent increase in total aberration frequency with increase in treatment concentration (Fig.54A App.VIA). The frequency of this parameter was lower than that induced by lasers exposures of 488 nm and 400 mW of 476 nm and similar to other laser exposures. In *V. faba* also, HA treatment induced dose dependent increase in total aberration frequency with increase in HA concentration (Fig.63B App.VIIB). The frequency of this parameter was lower than that induced by laser exposures of 400 mW of 488 nm and similar to other laser exposures.

A comparison of the percentage of total mitotic aberration in *A. cepa* and *V. faba* indicates that laser exposures of 476 and 488 nm induced dose dependent increase while 514 nm induced no dose dependence in total aberration frequency in *A. cepa* but in *V. faba* laser exposures of 476 and 514 nm induced no dose dependent change in the frequency of total aberration. UV exposure induced dose dependent increase in *A. cepa* but dose dependent decrease in *V. faba* in total aberration frequency. EMS and HA treatments caused dose dependent increase in total aberration frequency in both plant species.

4.2.6 Mitotic index

Mitotic index is used as a measure to denote the number of cells undergoing mitotic cell division. Mitotic index was found to be highest in control in both plant species as compared to the mutagen treated samples.

In *A. cepa,* laser exposures of 476 nm induced decrease in mitotic index with increase in time of exposure but the index was not dependent on the power density (Fig.55A App.IA). However laser exposures at 488 and 514 nm induced dose dependent decrease in mitotic index with increase in exposure time and power density (Fig.56A and 57A App.IIA and IIIA). The mitotic index at 476 nm and 200 and 400 mW of 514 nm were similar but higher than that of other laser exposures.

In *V. faba,* the mitotic index was independent of power density in laser exposures of 476 nm. A decrease in mitotic index with increase in exposure time was observed in 200 mW (Fig.64B App.IB) : 488 and 514 nm laser exposures induced dose dependent decrease in mitotic index with increase in power density and exposure time (Figs.65B and 66B App.IIB and IIIB).

In *A. cepa,* UV exposures did not induce a dose related change in mitotic index (Fig.58A App.IVA). The mitotic index of UV treated samples was similar to that in samples exposed to lasers of 476 nm and 514 nm at power densities 200 and 400 mW and higher than that of 488 and 600 and 800 mW of 514 nm exposure. In *V. faba* also no dose dependence in mitotic index was noticed in UV treated samples (Fig.67B App.IVB). The mitotic index was slightly higher than that caused by laser exposures.

γ - irradations induced dose dependent decrease in mitotic index in *V. faba* with increase in irradiation dose (Fig.68B App.VB). The mitotic index was similar to that induced by laser exposures.

EMS treatments in *A. cepa* caused dose dependent decrease in mitotic index with increase in concentration (Fig.59A App.VA). Mitotic index induced by laser of 476 and 200 and 400 mW of 514 nm were higher and 488

Fig. 55 A : Effect of laser exposure at 476 nm on mitotic indices in *A. cepa*

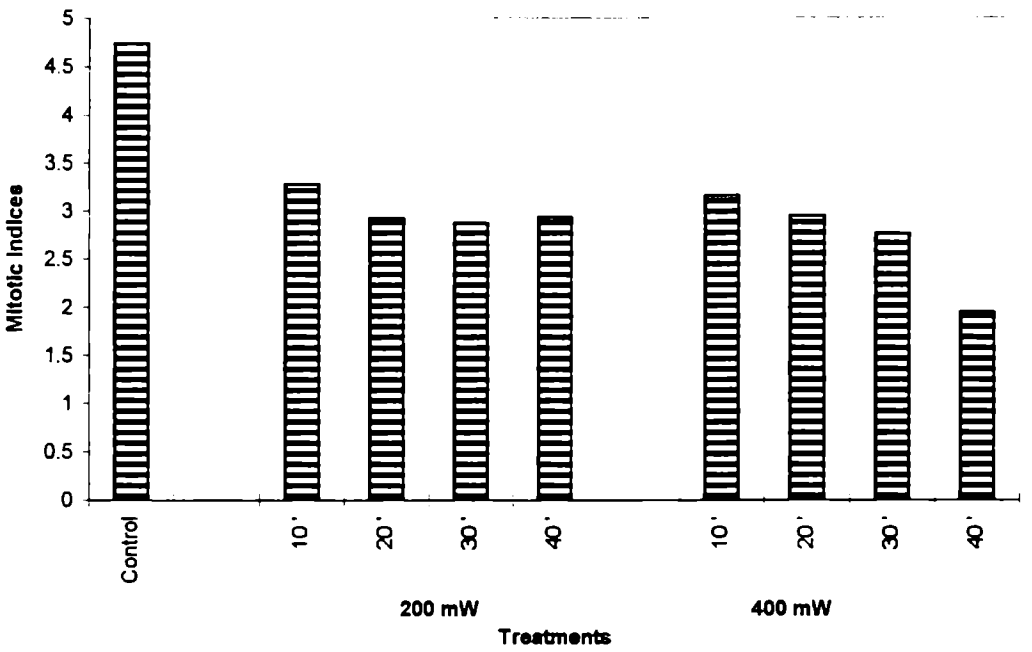


Fig. 56 A : Effect of laser exposure at 488 nm on mitotic indices in *A. cepa*

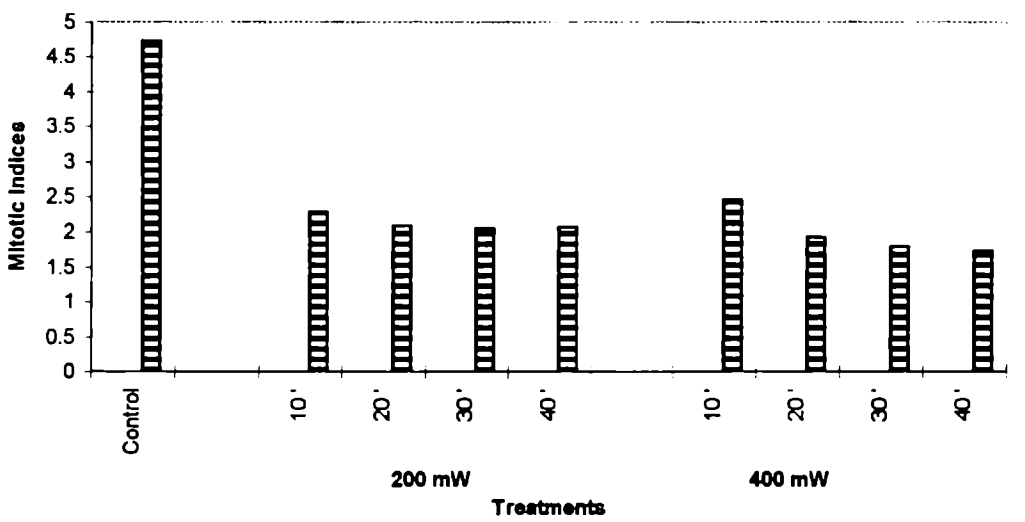


Fig. 57 A : Effect of laser irradiation at 514 nm on mitotic index in *A. cepa*

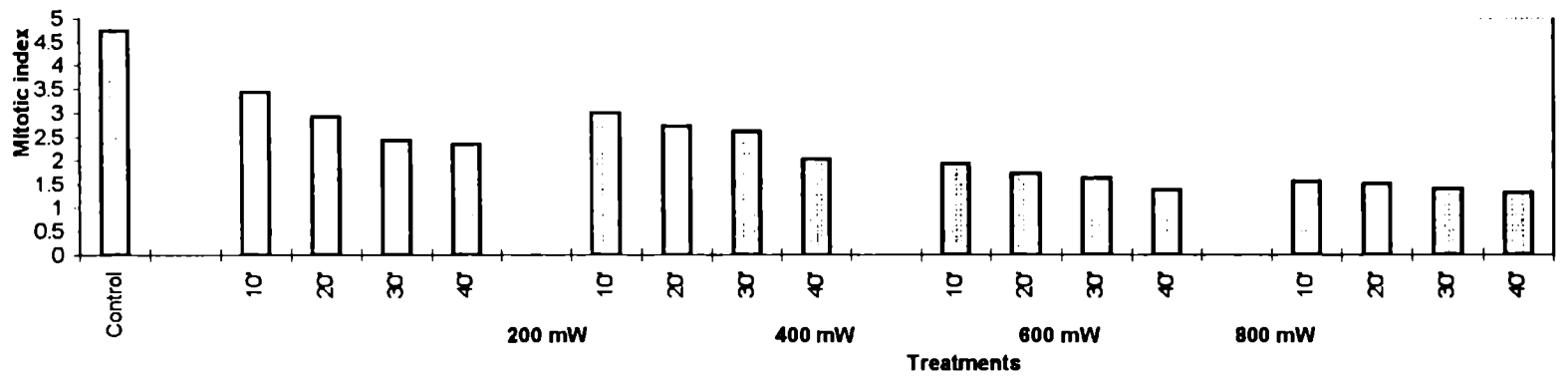


Fig. 58 A : Effect of UV exposure on mitotic indices in *A. cepa*

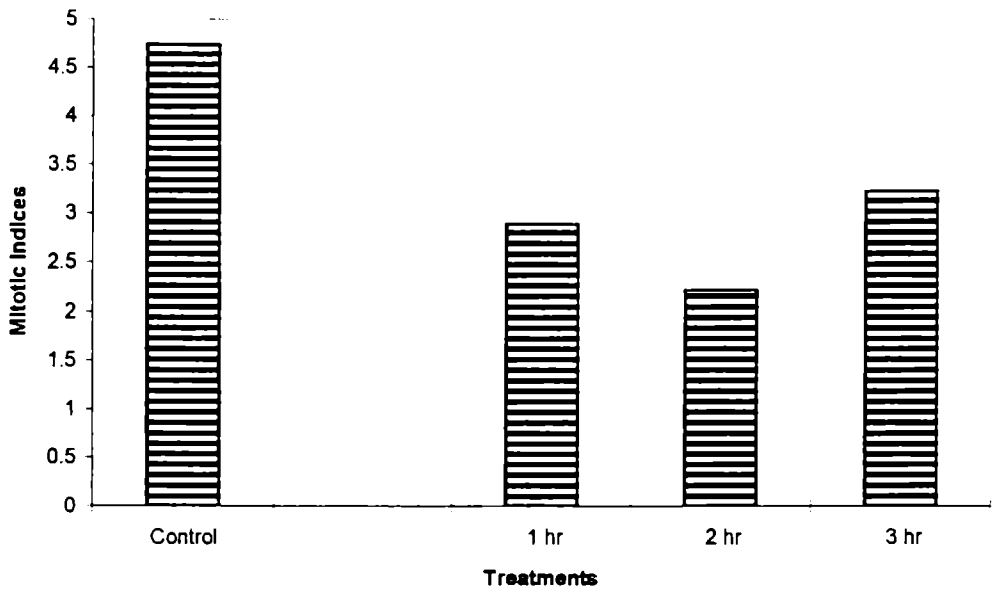


Fig. 59 A : Effect of EMS treatment on mitotic indices in *A. cepa*

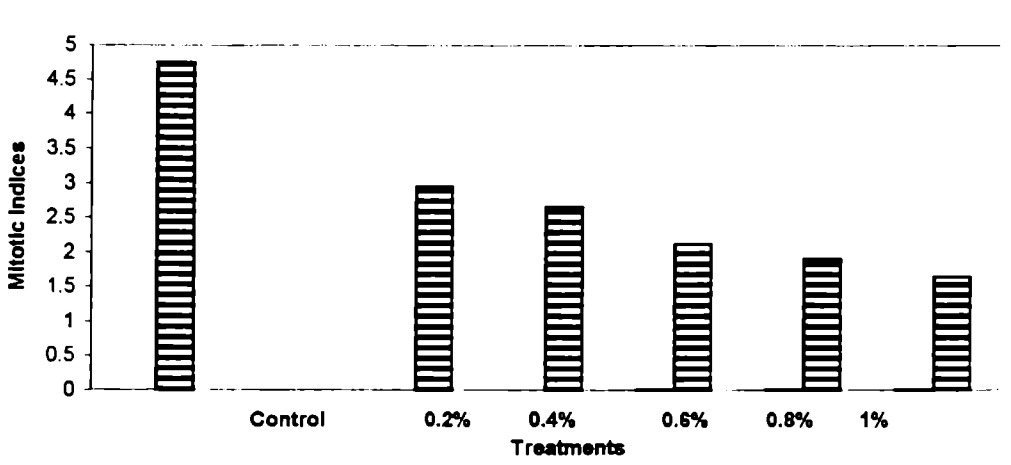


Fig. 60 A : Effect of HA treatment on mitotic indices in *A. cepa*

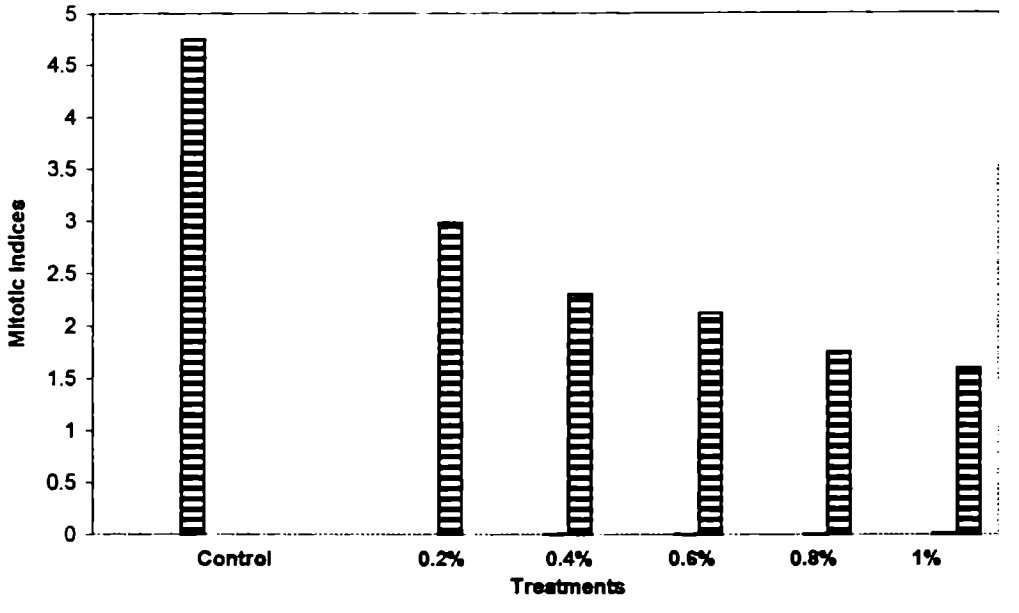


Fig. 64 B : Effect of laser exposure at 476 nm on mitotic indices in *V. faba*

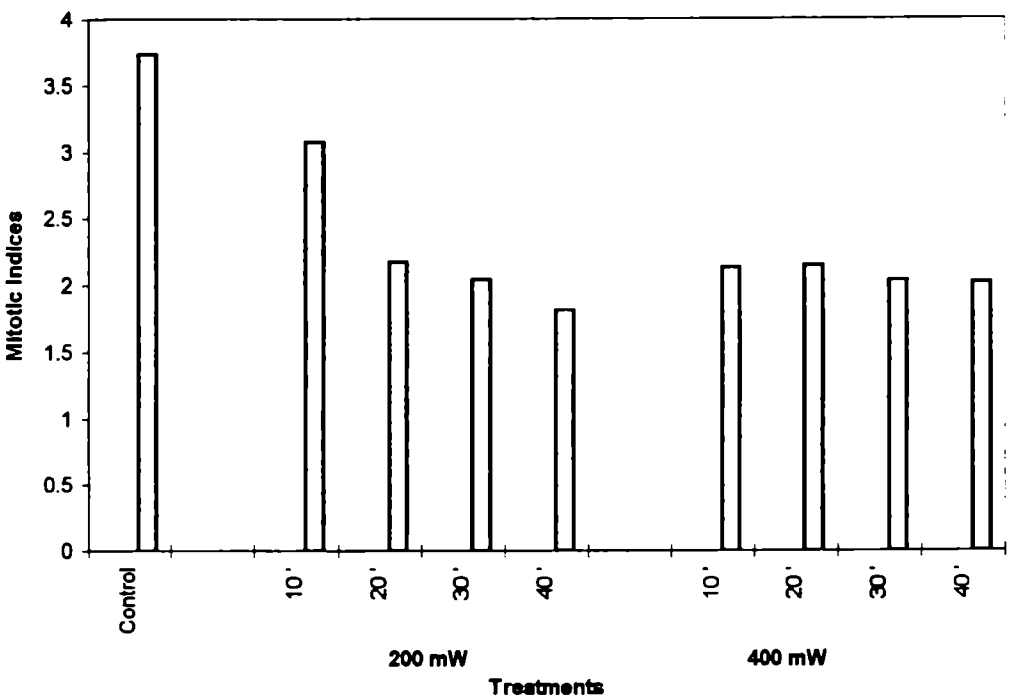


Fig. 65 B : Effect of laser exposure at 488 nm on mitotic indices in *V. faba*

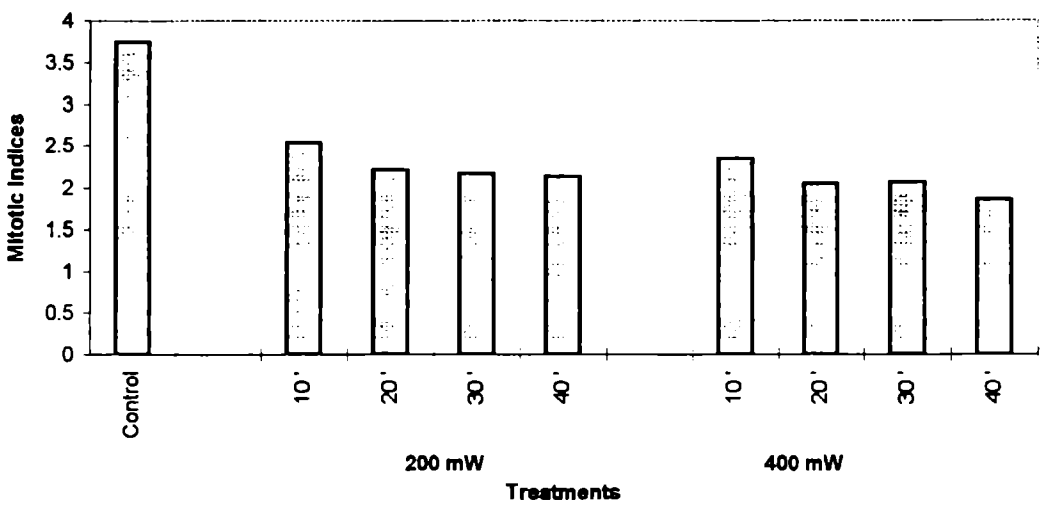


Fig. 66 B : Effect of laser irradiation at 514 nm on mitotic index in *V. faba*

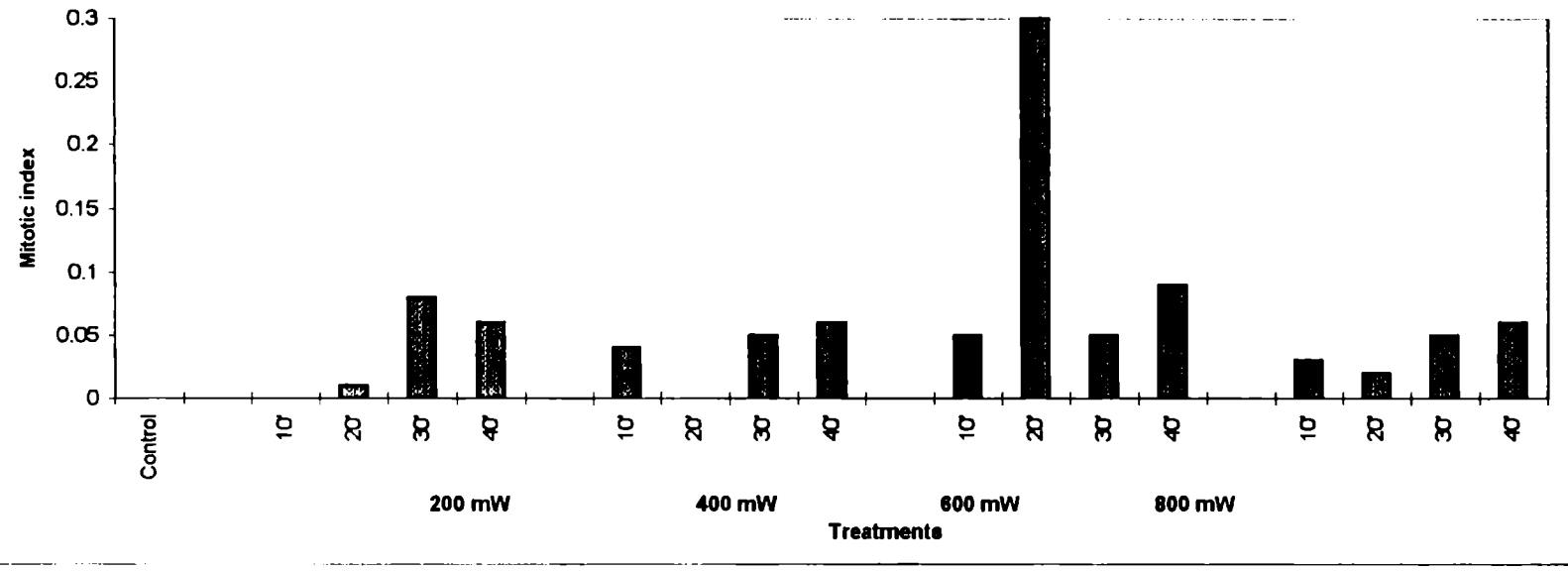


Fig. 67 B : Effect of UV exposure on mitotic indices in *V. faba*

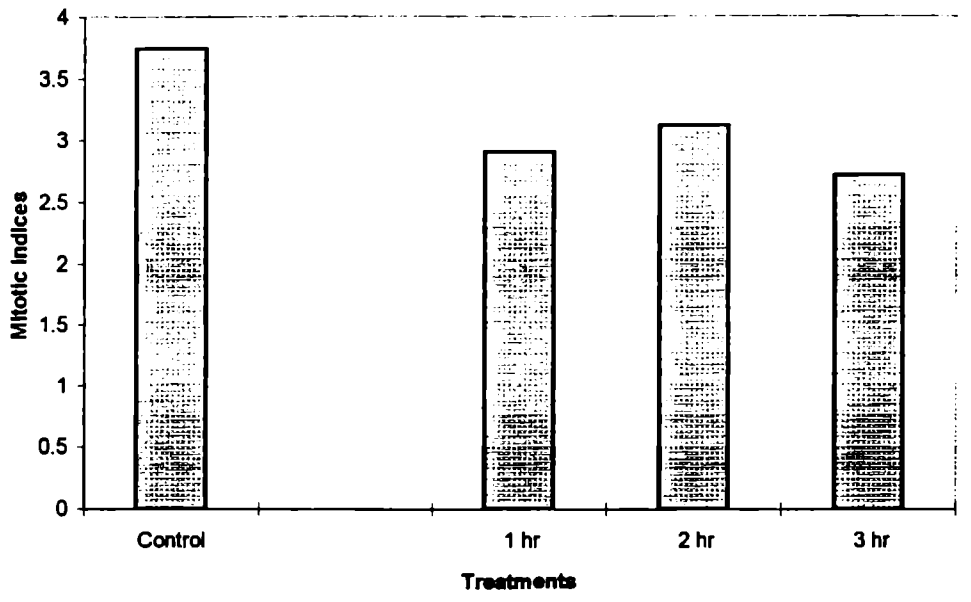


Fig. 68 B : Effect of gamma irradiation on mitotic indices in *V. faba*

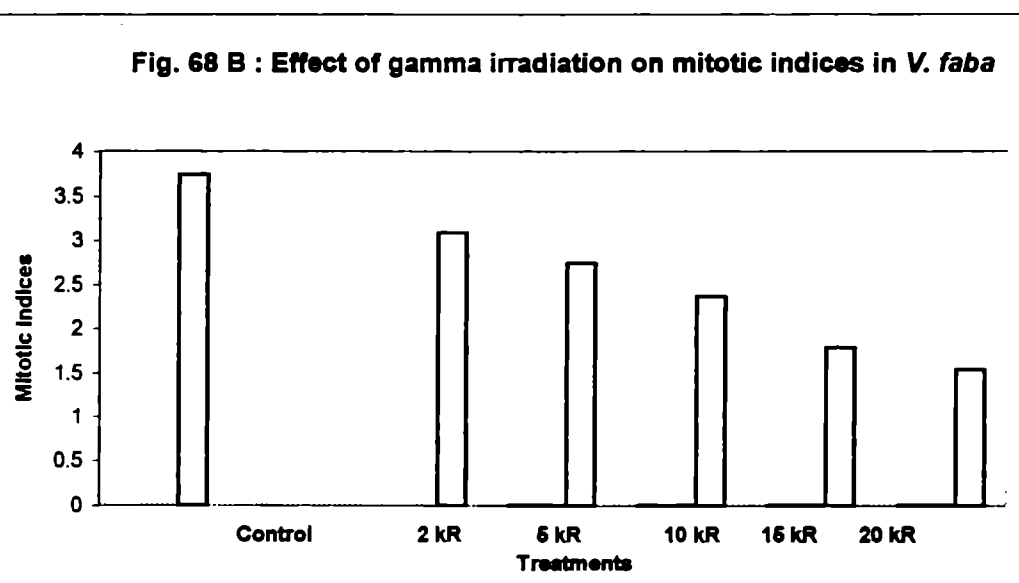


Fig. 69 B : Effect of EMS treatment on mitotic indices in *V. faba*

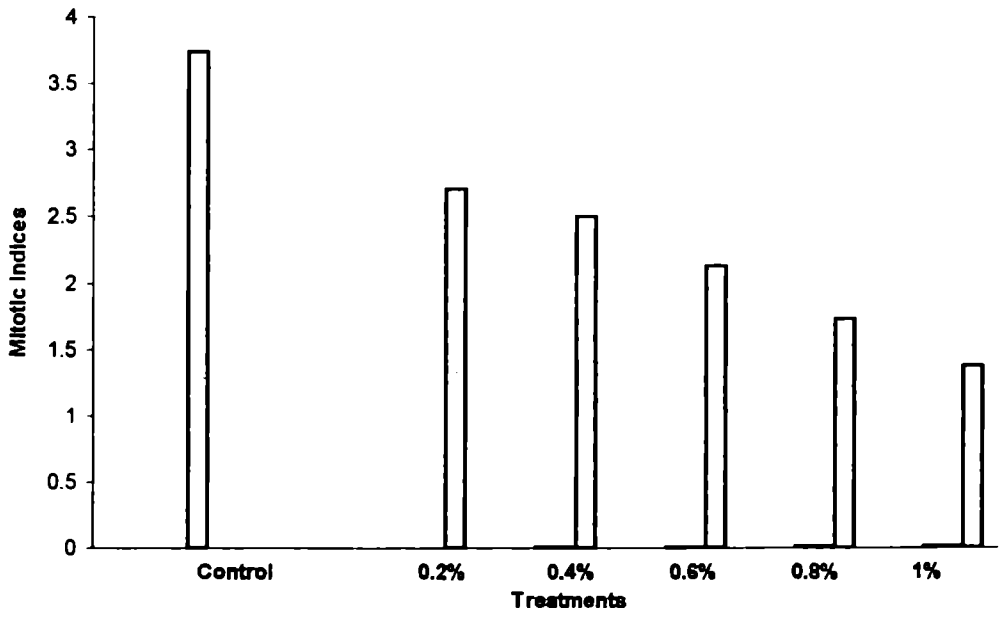


Fig. 70 B : Effect of HA treatment on mitotic indices in *V. faba*

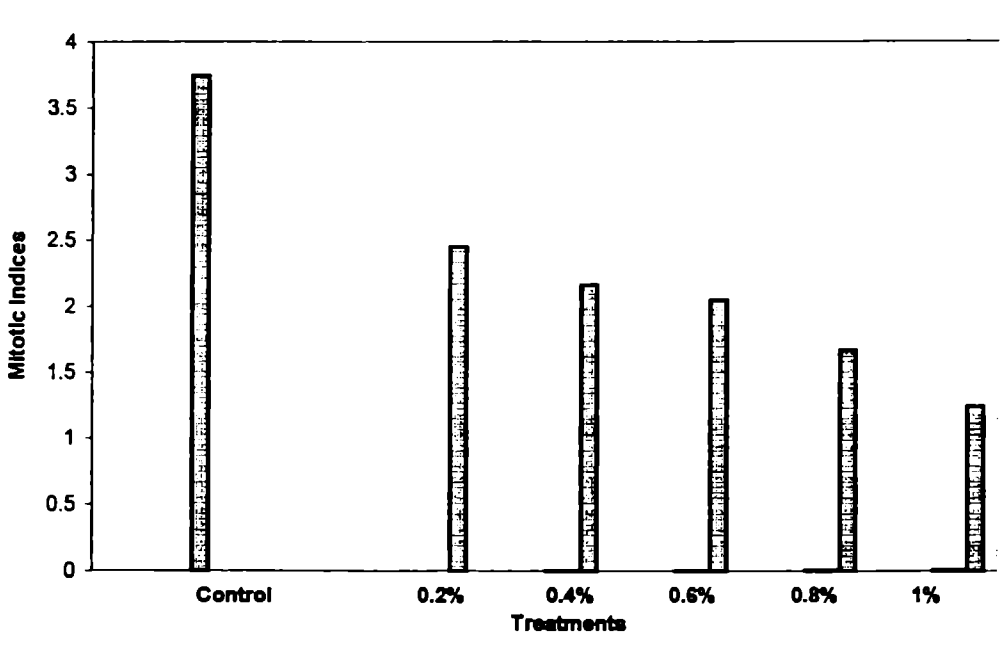
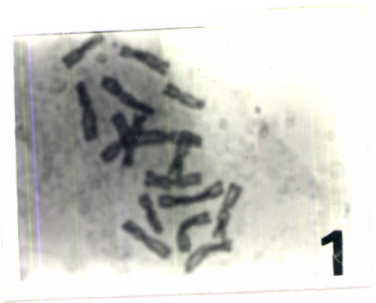


Fig. 1	:	*A. cepa* control with 16 chromosomes.
Figs. 2-7	:	Effect of laser exposure at 476 nm on mitosis in *A. cepa.*
Fig. 2	:	Metaphase with nondisjunction of chromosomes.
" 3	:	Metaphase with chromosome rings.
" 4		Star shaped anaphase with bridges.
" 5	:	Anaphase with multiple bridges.
" 6		Anaphase with multiple bridges and laggards.
" 7	:	Telophase with highly elongated strap shaped nucleus.
Figs. 8-17		Effect of laser exposure at 488 nm on mitosis in *A. cepa.*
Fig. 8	:	Prophase with clumping of chromosomes.
" 9	:	Metaphase with chromosome ring.
" 10	:	Metaphase with chromatid ring.
" 11	:	Metaphase with two translocation chromosomes and acentric fragments
" 12		Star shaped anaphase and bridge
" 13	:	Anaphase with single bridge.
" 14	:	Anaphase with multiple bridge.
" 15		Telophase with highly elongated strap shaped nuclei.
" 16		Nuclear polymorphism.
" 17		Telophase with micronucleate cells. (X 1000)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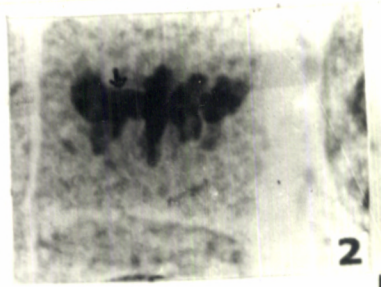
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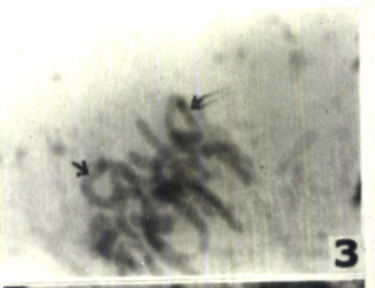
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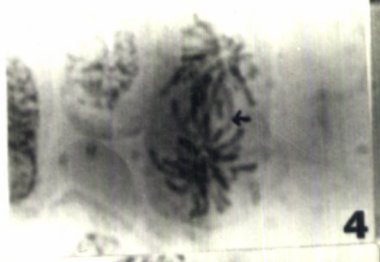
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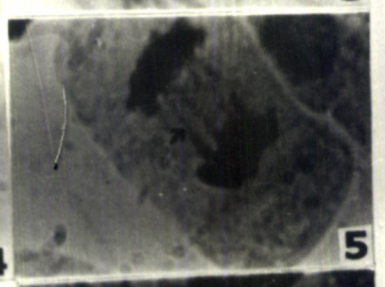
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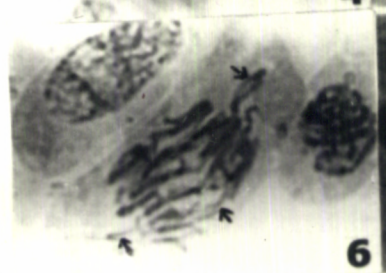
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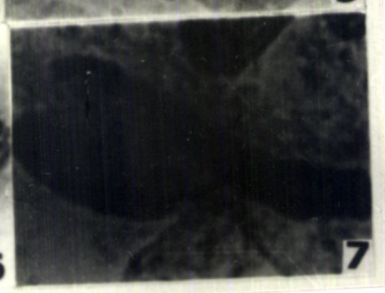
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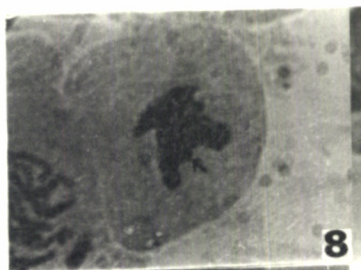
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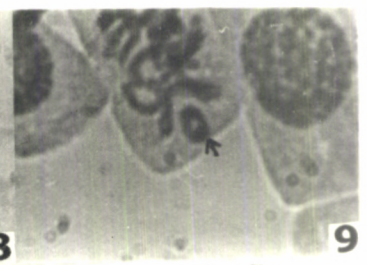
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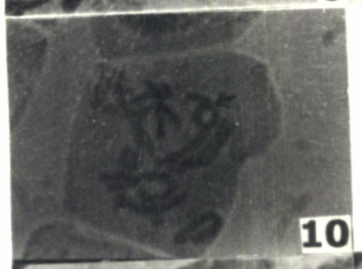
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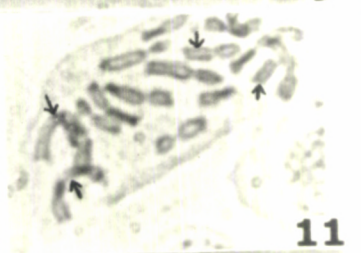
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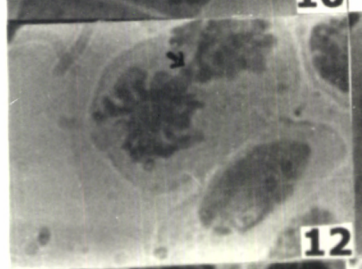
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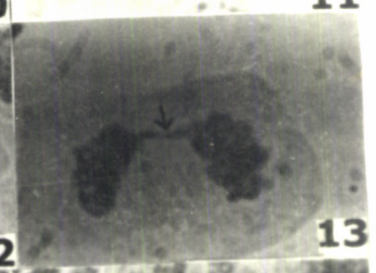
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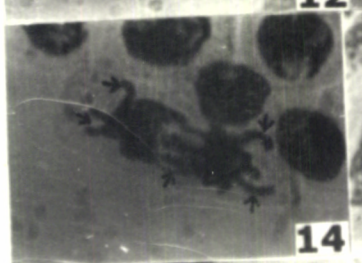
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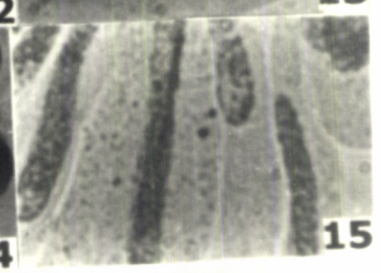
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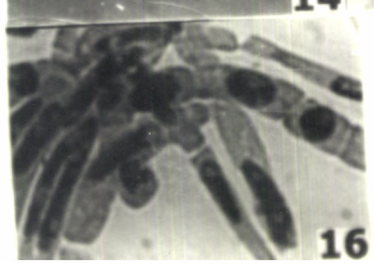
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Figs. 18-29 : Effect of laser exposure at 514 nm on mitosis in *A. cepa.*

Fig. 18 : Metaphase with nondisjunction of chromosomes.

" 19 : Metaphase with tetracentric chromosome and chromatid ring.

" 20 Tetraploid cell.

" 21 Metaphase with chromatid ring.

" 22 : Metaphase with disturbed polarity and chromatin droplets.

" 23 : Star shaped anaphase with chromosome bridge and tripolarity.

" 24 : Anaphase with two unequal bridges.

" 25 : Anaphase with multiple bridges and laggards.

" 26 : Telophase with late nucleus formation in one pole.

" 27 : Cells with chromatin droplets.

" 28 : Interphase cell with two micronuclei.

" 29 Nuclear polymorphism

Figs. 30 - 37 : Effect of EMS on mitosis in *A. cepa*

" 30 : Metaphase with chromosome ring

" 31 : Anaphase with lagging acentric fragments

" 32 : Anaphase with single bridge

" 33 : Anaphase with multiple bridges and lagging chromosome ring

" 34 : Anaphase with multiple bridges and lagging chromosome rings

" 35 : Anaphase with multiple bridges and laggards

" 36 Telophase with binucleate cell

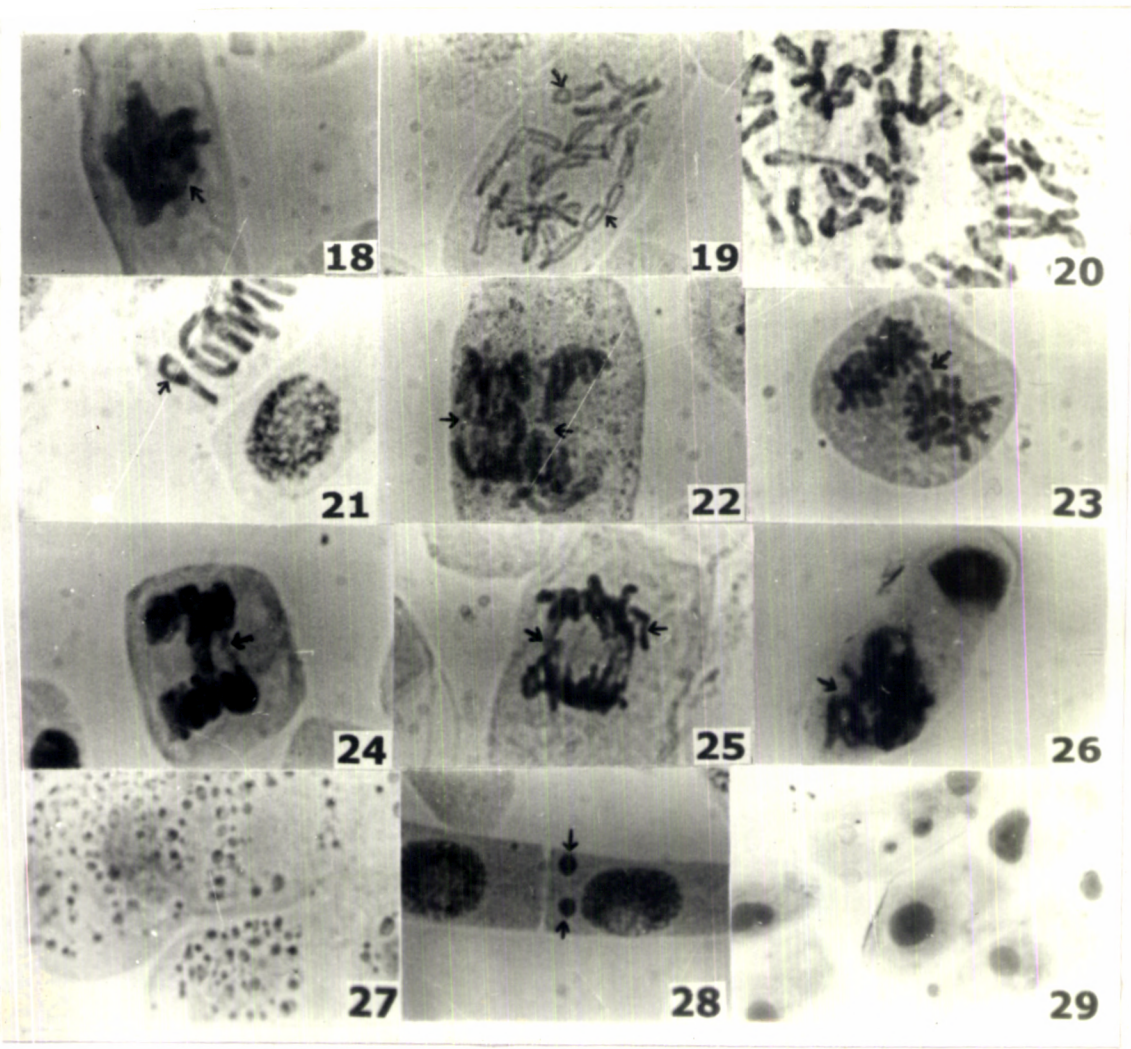
" 37 : Telophase with highly elongated strap shaped nuclei

Figs. 38 to 41 : Effect of HA treatment on mitosis in *A. cepa*

Fig. 38 : Metaphase with chromosome ring

Figs. 39 - 41 : Anaphase with multiple bridges and laggards

Fig. 42 : Sterile pollen grain in *A. cepa* (X 1000)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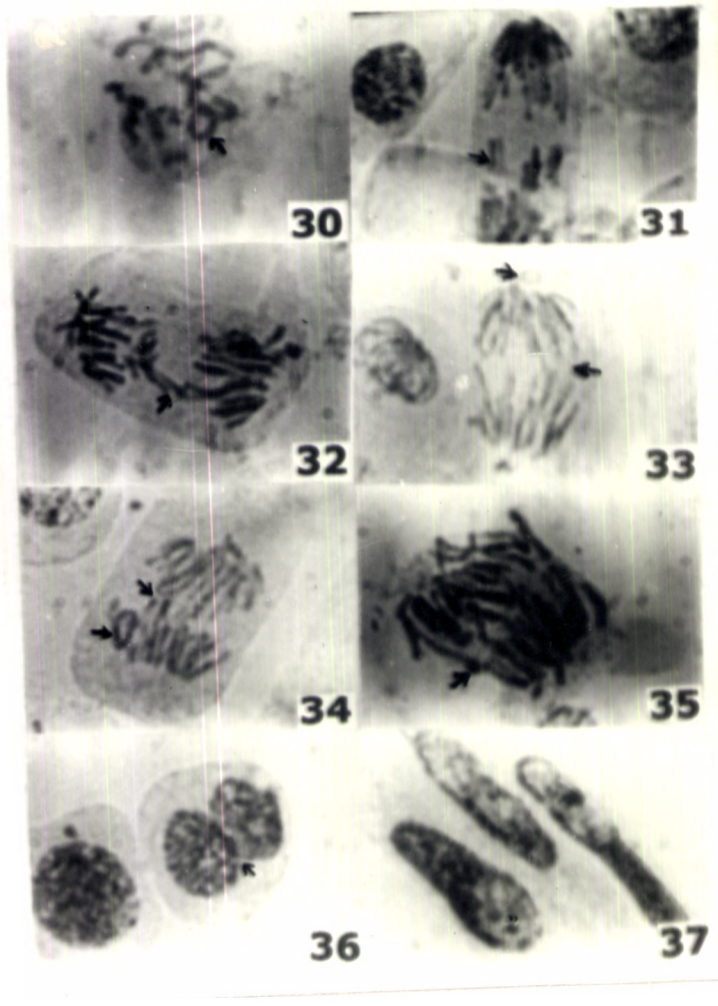
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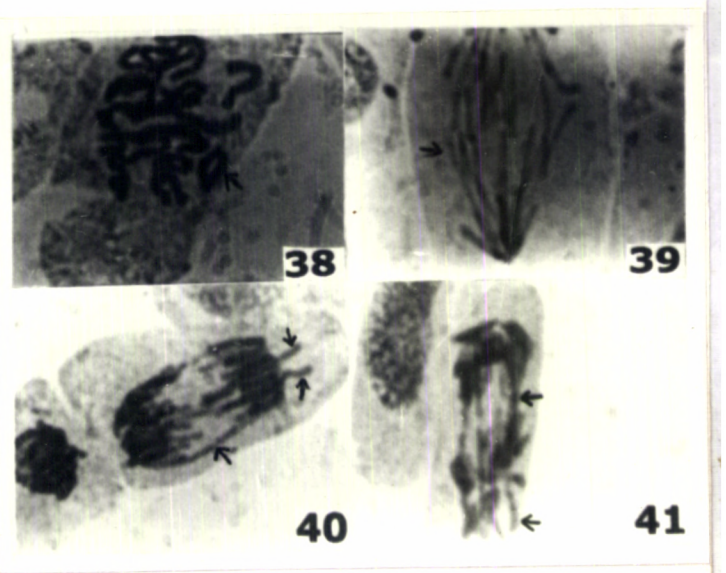
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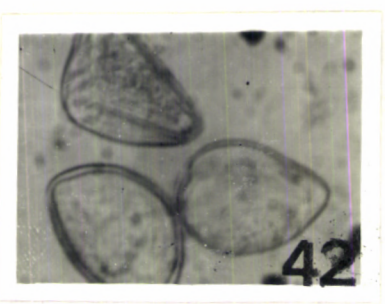
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Fig. 43 : *V. faba* control with 12 chromosomes

Figs. 44 - 47 : Effect of laser exposure at 476 nm on mitosis in *V. faba*

Fig. 44 : Metaphase with chromosome ring

" 45 :Polyploid cell (octaploid)

Figs. 46, 47 :Telophase with highly elongated strap shaped nuclei (X 1000)

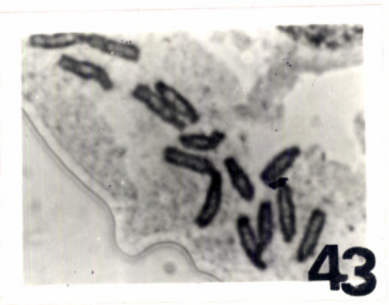
Fig. 43 : *V. faba* control with 12 chromosomes

Figs. 44 - 47 : Effect of laser exposure at 476 nm on mitosis in *V. faba*

Fig. 44 : Metaphase with chromosome ring

" 45 :Polyploid cell (octaploid)

Figs. 46, 47 :Telophase with highly elongated strap shaped nuclei (X 1000)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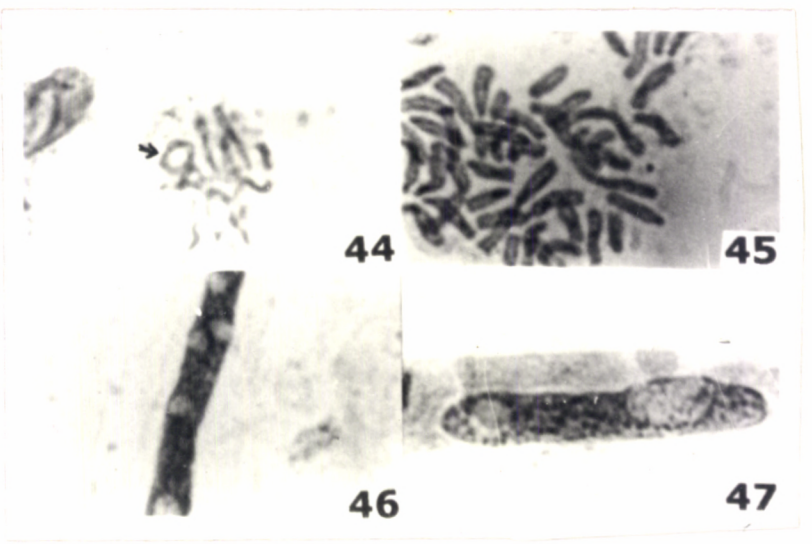
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Figs. 48 - 65 : Effect of laser exposure at 488 nm on mitosis in *V. faba*

Fig. 48 : Prophase with clumping of chromosomes

" 49 : Metaphase with nondisjunction of chromosomes and chromosome ring

" 50 : Metaphase with nondisjunction of chromosomes

" 51 : Metaphase with tricentric chromosome

" 52 : Metaphase with tetracentric chromosome

" 53 : Metaphase with late condensation in 3 chromosomes

" 54 : Metaphase with chromosome ring

" 55 : Metaphase with chromatid ring

" 56 : Anaphase with single bridge

Figs. 57 - 59 : Anaphase with multiple bridges and laggards

Fig. 60 : Cell with chromatin droplets

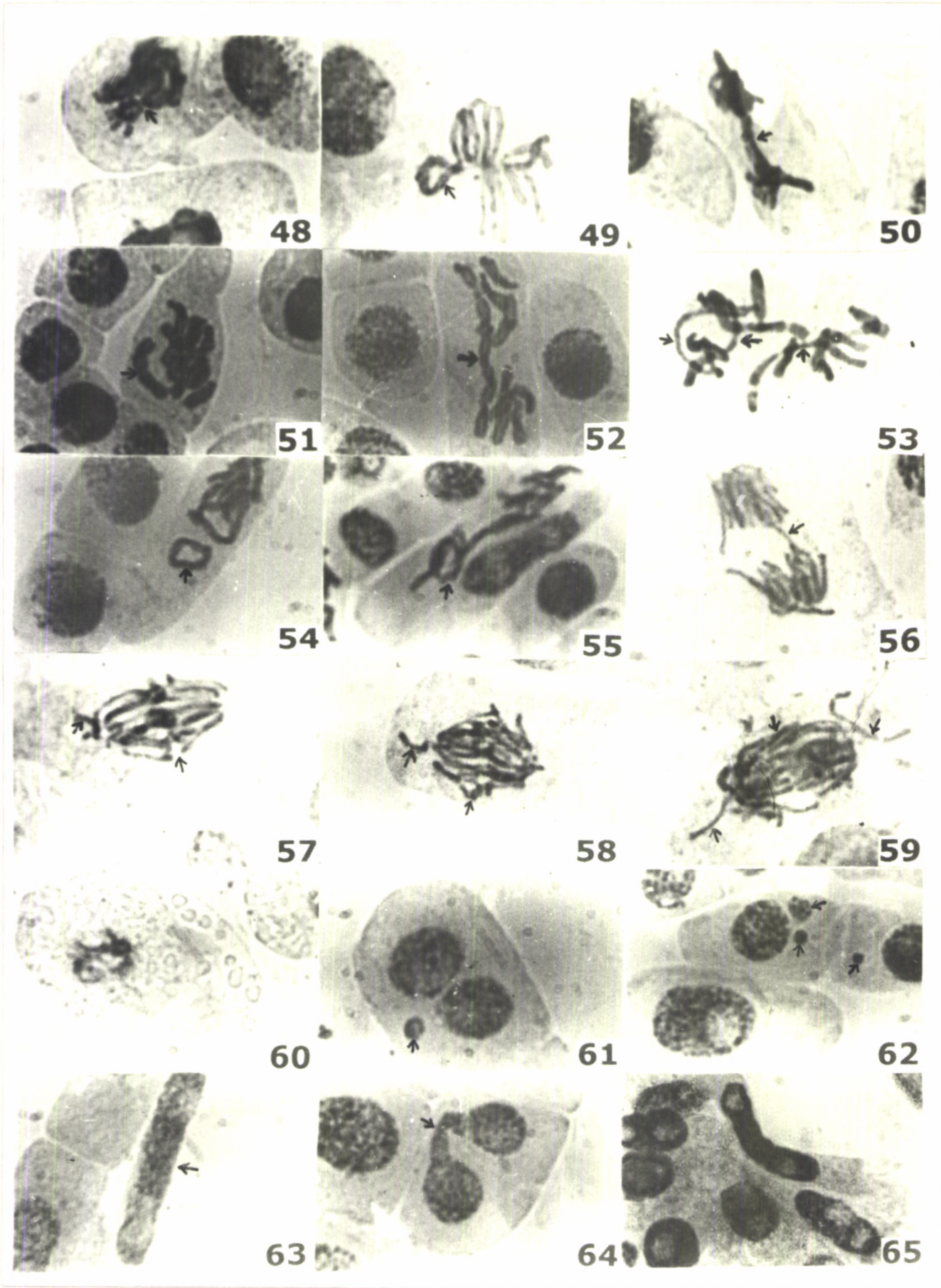
" 61 : Telophase with binucleate cell and micronucleus

" 62 : Telophase with micronuclei

" 63 : Telophase with highly elongated strap shaped nucleus

" 64 : Telophase with nuclear bridge

" 65 : Nuclear polymorphism (X 1000)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Figs. 66 - 80 : Effect of laser exposure at 514 nm on mitosis in *V. faba*

Fig. 66 : Prophase with clumping of chromosomes

" 67 : Metaphase with nondisjuntion of chromosomes

" 68 : Metaphase with dicentric chromosome

" 69 : Metaphase with tricentric chromosome

" 70 : Metaphase with chromosome ring

" 71 : Metaphase with chromatid ring

" 72 : Disturbed metaphase with chromatin droplets

" 73 : Tetraploid cell

" 74 : Anaphase with multiple bridges

" 75 : Telophase with lagging acentric fragment

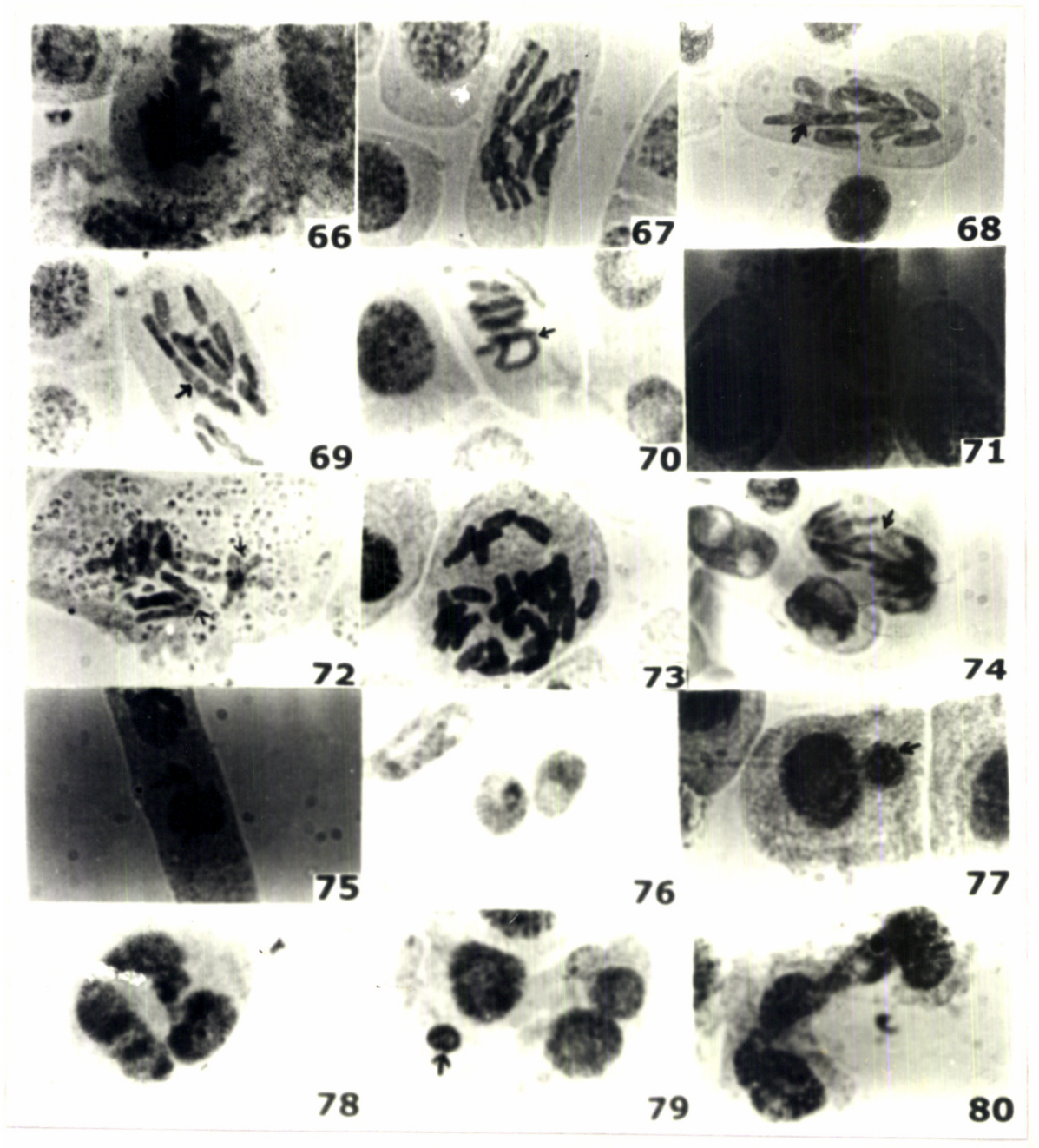
" 76 : Telophase with binucleate cell

" 77 : Telophase with micronucleus

" 78 : Interphase with an unequal distribution of genetic material shown by the presence of 3 different sized nuclei

" 79 : Interphase with an unequal distribution of genetic material shown by the presence of 4 different sized nuclei associated with 1 micronucleus in a cell which has just undergone cell cleavage giving rise to 2 different sized daughter cells

" 80 : Interphase with an unequal distribution of genetic material shown by an irregular shaped nucleus (X 1000)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81 - 88 : Effect of UV exposure on mitosis in *V. faba*

81 : Prophase with clumping of chromosomes.

82 : Metaphase with nondisjunction of chromosomes.

83 : Metaphase with chromosome ring.

84 : Metaphase with chromatid ring.

85 : Anaphase with lagging acentric fragments.

86 : Anaphase with bridge, laggard and tripolar orientation.

87 : Anaphase with multiple bridges and acentric fragments.

88 : Anaphase with only one pole.

89-100 : Effect of γ-irradiation on mitosis in *V. faba*

89 : Prophase with clumping of chromosomes.

90 : Metaphase with nondisjunction of chromosomes.

91 : Early metaphase with chromosome ring.

92 : Metaphase with chromosome ring.

93 : Early metaphase with chromatid ring.

94 : Metaphase with chromatid ring.

95 : Metaphase with dicentric chromosome, isochromatid deletion and acentric fragments

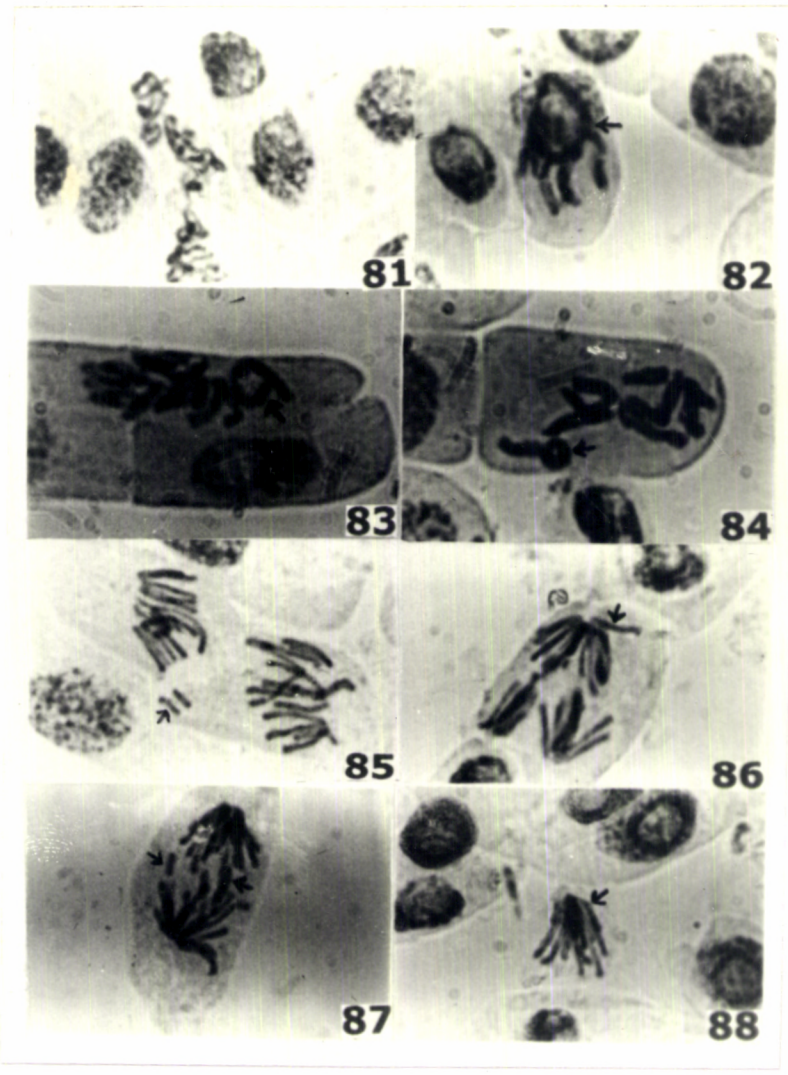
96 : Metaphase with dicentric chromosome and late condensation in one chromosome.

97 : Metaphase with dicentric and tricentric chromosomes.

98 : Metaphase with translocation chromosome, dicentric chromosome and minute.

99 : Metaphase with tricentric chromosome.

100 : Metaphase with tetracentric chromosome. (X 1000)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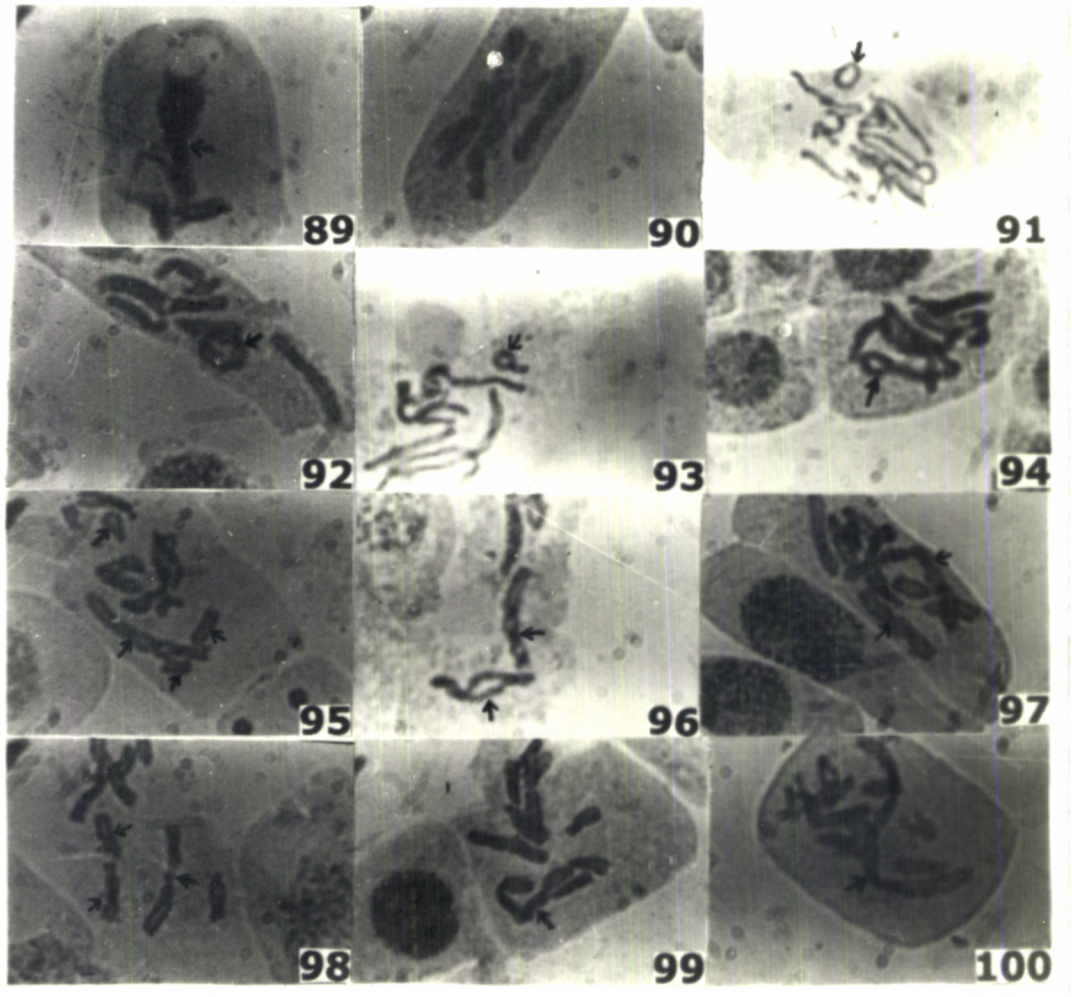
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Figs. 101-112 : Effect of γ-irradiation on mitosis in *V. faba*

Fig. 101 : Early metaphase with double minute.

" 102 : Metaphase with achromatic gap in one chromosome and chromatid ring.

" 103 : Metaphase with tetracentric chromosome.

" 104 : Tetraploid cell.

" 105 : Anaphase with bridge and laggards.

" 106 : Anaphase with bridge, lagging acentric fragment and lagging ring chromosome.

" 107 : Anaphase with bridge, lagging chromosomes and ring chromosomes.

" 108 : Chromatin droplets.

" 109 : Telophase with multi nucleate cell.

" 110 : Telophase with nuclear bridge and micronuclei.

Figs.111 and 112 : Telophase cells with micronuclei.

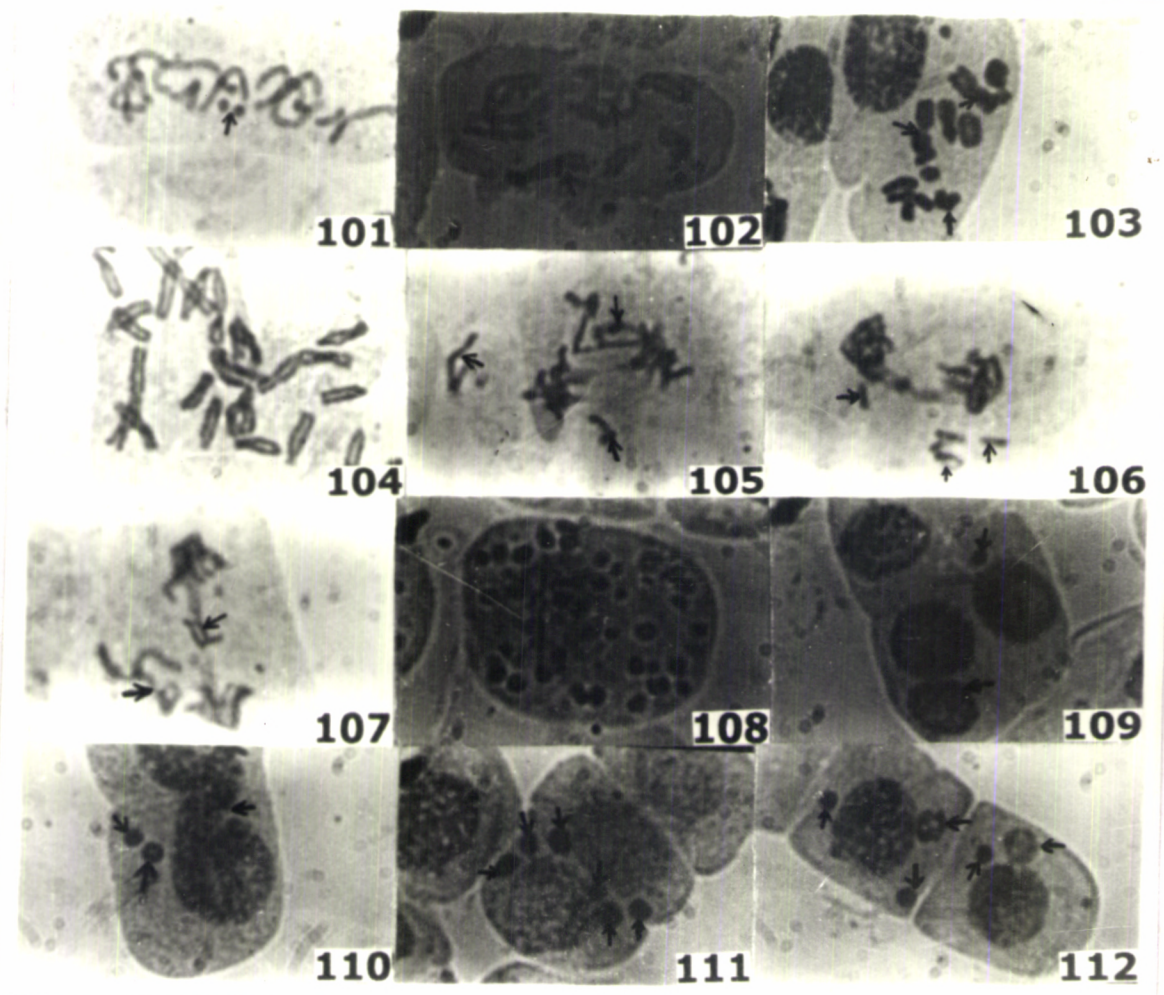
Figs. 113-116 : Effect of EMS on mitosis in *V. faba*

Fig. 113 : Metaphase with tricentric chromosome.

" 114 : Anaphase with lagging ring chromosome.

" 115 : Anaphase with multiple bridge.

" 116 : Telophase with binucleate cells. (X 1000)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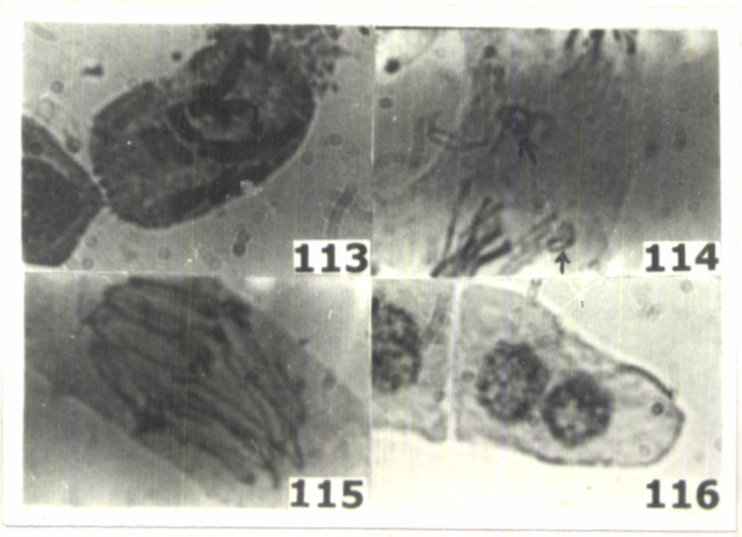
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nm and 600 and 800 mW of 514 nm were similar to that caused by EMS treatments. In *V. faba* also, EMS caused dose dependent decrease in mitotic index with increase in treatment concentration (Fig.69B App.VIB). The mitotic index was similar to that induced by laser exposures.

HA treatments in *A. cepa* caused dose dependent decrease in mitotic index with increase in mutagen concentration (Fig.60A App.VIA). The mitotic index was lower than that induced by 476 nm and 200 and 400 mW of 514 nm laser exposures and similar to 488 nm and 600 and 800 mW exposures of 514 nm laser exposures. In *V. faba* also HA treatment induced dose dependent decrease in mitotic index with increase in concentration (Fig.70B App.VIIB) and the mitotic index was similar to that caused by laser exposures.

By comparing the mitotic index in *A. cepa* and *V. faba*, it is evident that laser exposures of 488 and 514 nm induced dose dependent decrease in mitotic index in both plant species but 476 nm induced time dependent decrease in *A. cepa* and no dose dependence in *V. faba* in mitotic index. UV exposures did not cause dose dependent change in mitotic index in both plant species. EMS and HA treatments caused dose dependent decrease in mitotic index in both plant species.

4.3 Meiotic studies

A. cepa used for the present study was characterised by fully sterile pollen grains and no meiotic divisions were observed in pollen mother cells. Hence the meiotic and pollen stainability studies were not possible in this plant species.

v. rapa produced several types of meiotic aberrations in mutagen treatments. The important types were clumping and stickiness of chromosomes, bridges, laggards, unequal distribution of chromosomes and micronuclei. Very low frequency of these aberrations were observed in the control (untreated) samples (App.VIIIB).

4.3.1 Clumping of chromosomes

The aberration mainly seen at prophase - 1 and metaphase -1 in mutagen treated samples was the clumping of chromosomes (plate figs.118, 120, 121). This change was rarely observed in control (untreated) samples.

Laser exposures of 476 and 514 nm induced a dose dependent increase (Fig.71B App.VIIIB) while laser exposure of 488 nm caused a dose dependent decrease in clumping frequency (Fig.71B App.VIIIB). The frequency of this change induced by laser exposure of 488 nm was higher than that induced by 476 but lower than that induced by 514 nm.

UV exposures also induced dose dependent increase in clumping dose dependent increase in clumping frequency with increase in exposure time (Fig.71B App.VIIIB). The frequency of this aberration induced by UV was lower than that induced by lasers at 488 nm but higher than that induced by 476 and 514 nm lasers.

γ - irradiations caused dose dependent increase in clumping frequency with increase in irradiation dose (Fig.71B App.VIIIB). The frequency of chromosome clumping induced by 5, 10 and 15 kR was higher than that caused by lasers.

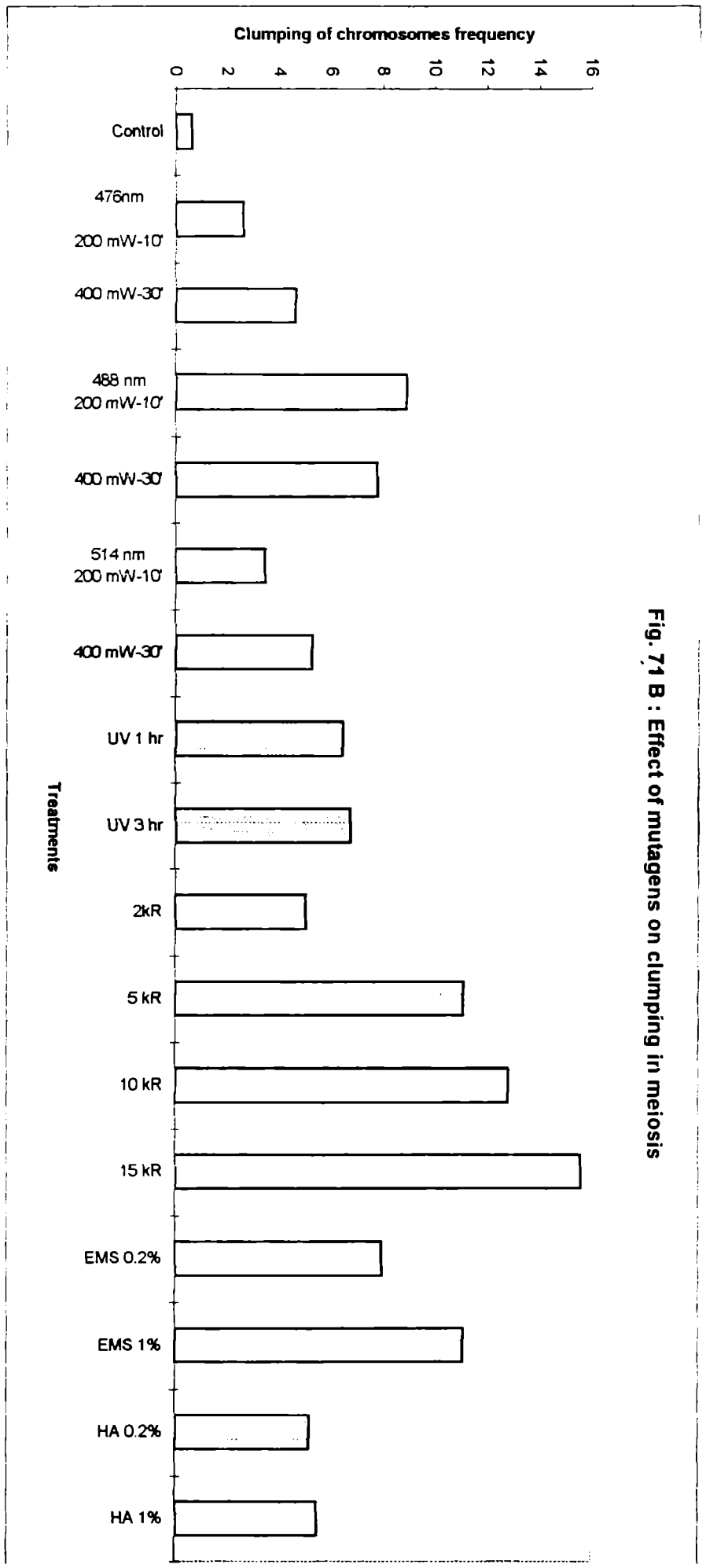


Fig. 71 B : Effect of mutagens on clumping in meiosis

EMS caused dose dependent increase in frequency of chromosome clumping with increase in treatment concentration (Fig.71B App.VIIIB). The clumping frequency induced by 1% EMS concentration was higher than that caused by laser exposures and by 0.2% similar to 488 nm and higher than 476 and 514 nm laser exposures.

HA also caused dose dependent increase in clumping frequency with increase in treatment concentration (Fig.71B App.VIIIB) and the frequencies were similar to that induced by lasers of 514 nm but lower than that induced by 488 nm and higher than that caused by 476 nm.

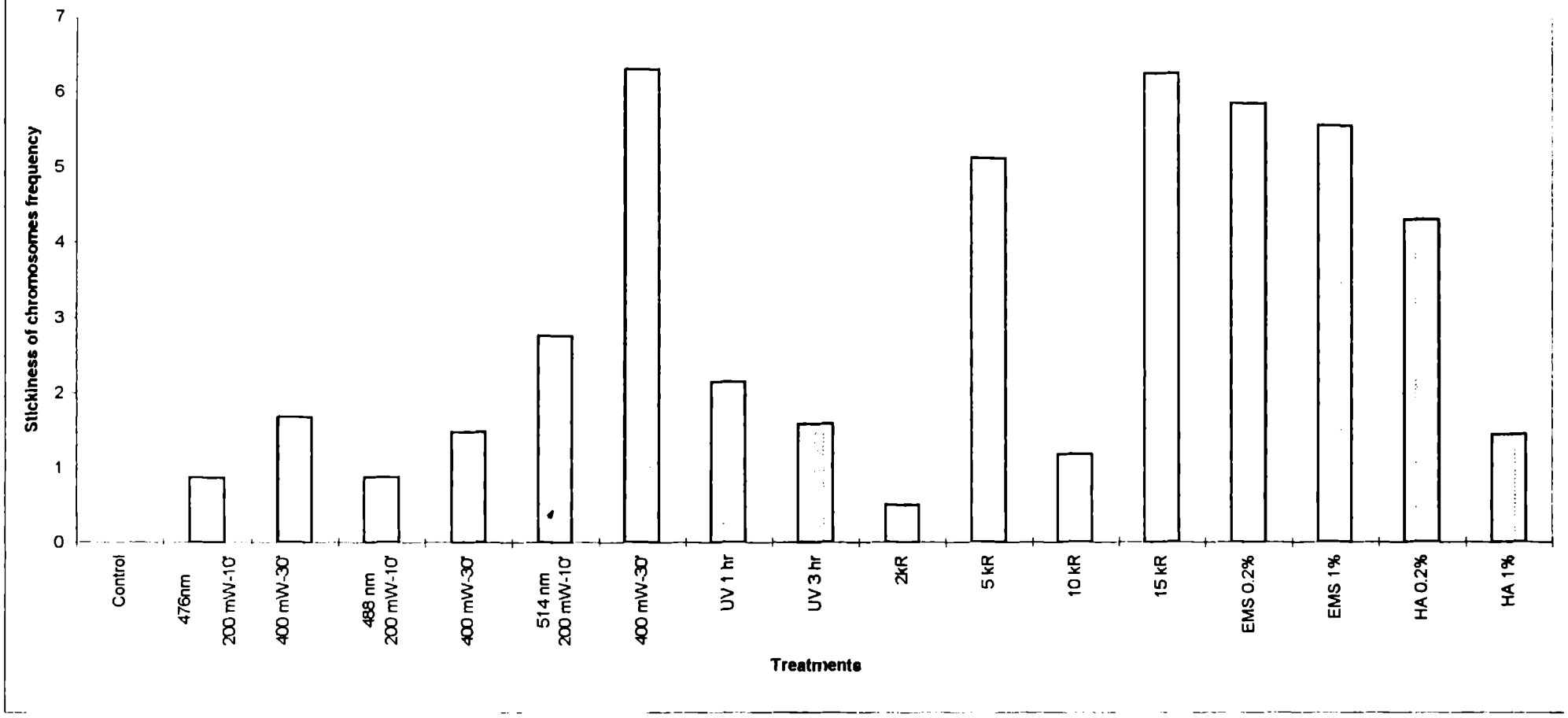
4.3.2 Stickiness

Another type of aberration mainly observed at diakinesis and metaphase stage of meiosis was stickiness of chromosomes (plate figs.119, 122). This aberration was not found in control.

Laser exposures of 476, 488 and 514 nm induced dose dependent increase in stickiness frequency with increase in power density (Fig.72B App.VIIIB). Stickiness frequency induced by 514 nm was higher than that induced by 476 and 488 nm. The frequency of this parameter was similar in laser exposures of 476 and 488 nm. Stickiness induced in exposure of 30 min in the power density of 400 mW of 514 nm was several fold higher than that induced by other laser exposures.

UV caused dose dependent decrease in stickiness (Fig.72B App.VIIIB) and the frequency of this change was lower than that induced by lasers of 514 nm and similar to that induced by 476 and 488 nm of laser exposures.

Fig. 72 B : Effect of mutagens on stickiness of chromosomes in meiosis



γ -irradiations induced dose dependent increase in stickiness frequency with increase in irradiation dose (Fig.72B App.VIIIB). The frequency of this aberration induced by 2 kR was found to be lower than that induced by laser exposures. The stickiness induced by 15 kR of γ -irradiation and that induced by 30 min exposure in the power density at 400 mW of 514 nm laser exposure were similar. Whereas 5,10 and 15 kR of γ -irradiations induced stickiness frequencies higher than that induced by laser exposures of 476 nm, 488 nm and in the power density 200 mW of 514 nm.

EMS induced dose dependent decrease in stickiness frequency with increase in treatment concentration (Fig.72B App.VIIIB) The frequency of this change induced by EMS was lower than that induced by laser exposure of 30 min in power density of 400 mW of 514 nm but was higher than the frequencies induced by other doses of lasers.

HA treatment caused dose dependent decrease in stickiness frequency with increase in treatment concentration (Fig.72B App.VIIIB). The frequency of this aberration in 0.2%. HA treatment was lower than that induced by laser exposure of 30 min in power density 400 mW of 514 nm but was higher than that induced by other laser exposures. 1% HA induced stickiness frequency lower than that caused by 30 min in power density 400 mW of 514 nm but similar to that induced by other laser exposures.

4.3.3 Bridges

Bridges were found at anaphase and telophase stages of meiotic cell division (plate figs.123, 124, 125, 127, 129). Bridges were not found in control and UV treatments. EMS of 1% concentration induced bridges with several fold higher frequency than the other treatments.

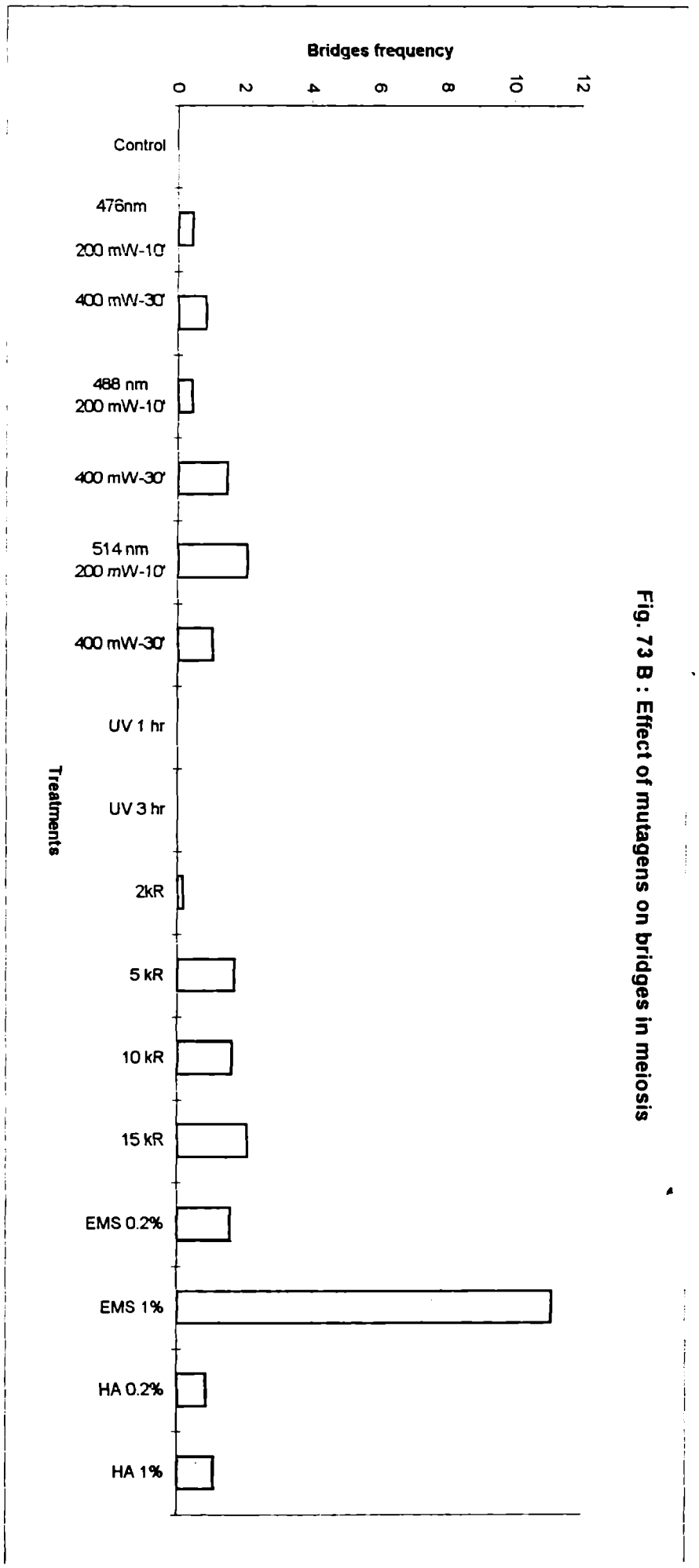


Fig. 73 B : Effect of mutagens on bridges in meiosis

Laser exposures of 476 and 488 nm induced dose dependent increase in bridge frequency with increase in power density (Fig.73B App.VIIIB) although laser at 514 nm caused dose dependent decrease in frequency (Fig.73B App.VIIIB). The frequency of bridges induced by lasers were found to be similar in all three wavelengths.

γ -irradiation induced dose dependent increase in bridge frequency with increase in irradiation dose (Fig.73B App.VIIIB). The frequency of this aberration induced by γ -irradiation of 2 kR was lower than that caused by laser exposures but the frequency induced by γ -irradiations of other doses were similar to that induced by laser exposures.

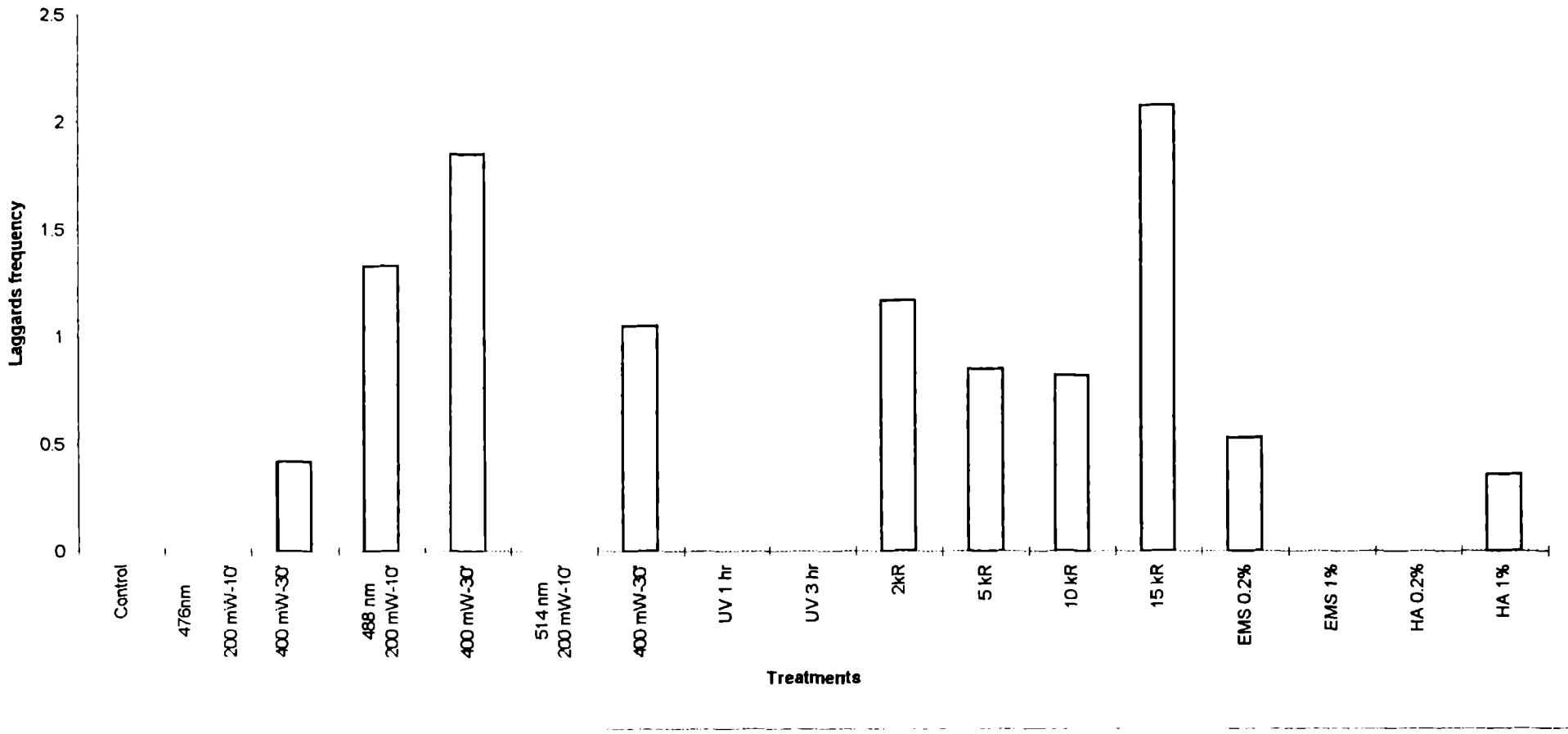
EMS also induced dose dependent increase in bridge frequency with increase in treatment concentration (Fig.73B App.VIIIB). The frequency of bridges induced by 1% concentration of EMS was several fold higher than that induced by laser exposures. The bridges induced by 0.2% of EMS treatment and that caused by lasers were found to be similar in frequency.

HA treatment induced dose dependent increase in frequency of this aberration with increase in treatment concentration (Fig.73B App.VIIIB). The frequency of bridges induced by HA treatments were similar to that induced by lasers.

4.3.4 Laggards

Laggards were found at anaphase and telophase stages of meiosis (plate figs. 131, 132). It was not found in certain mutagen treatments and in control.

Fig. 74 B : Effect of mutagens on laggards in meiosis



Laser exposures of 488 nm induced dose dependent increase in laggard frequency with increase in power density (Fig.74B App.VIIIB) but this aberration was induced by 476 and 514 nm only in 30 min exposures of power density 400 mW (Fig.74B App.VIIIB). The frequency of laggards induced by laser of 488 nm was higher than that induced by 476 and 514 nm.

Laggards were not found in samples exposed to UV radiations.

γ -irradiations induced dose dependent increase in laggard frequency with increase in treatment dose (Fig.74B App.VIIIB). The frequency of this aberration induced by 2kR of γ-irradiations was lower than that induced by laser exposures but in the other γ-irradiationγs laggard frequencies were similar to that induced by lasers.

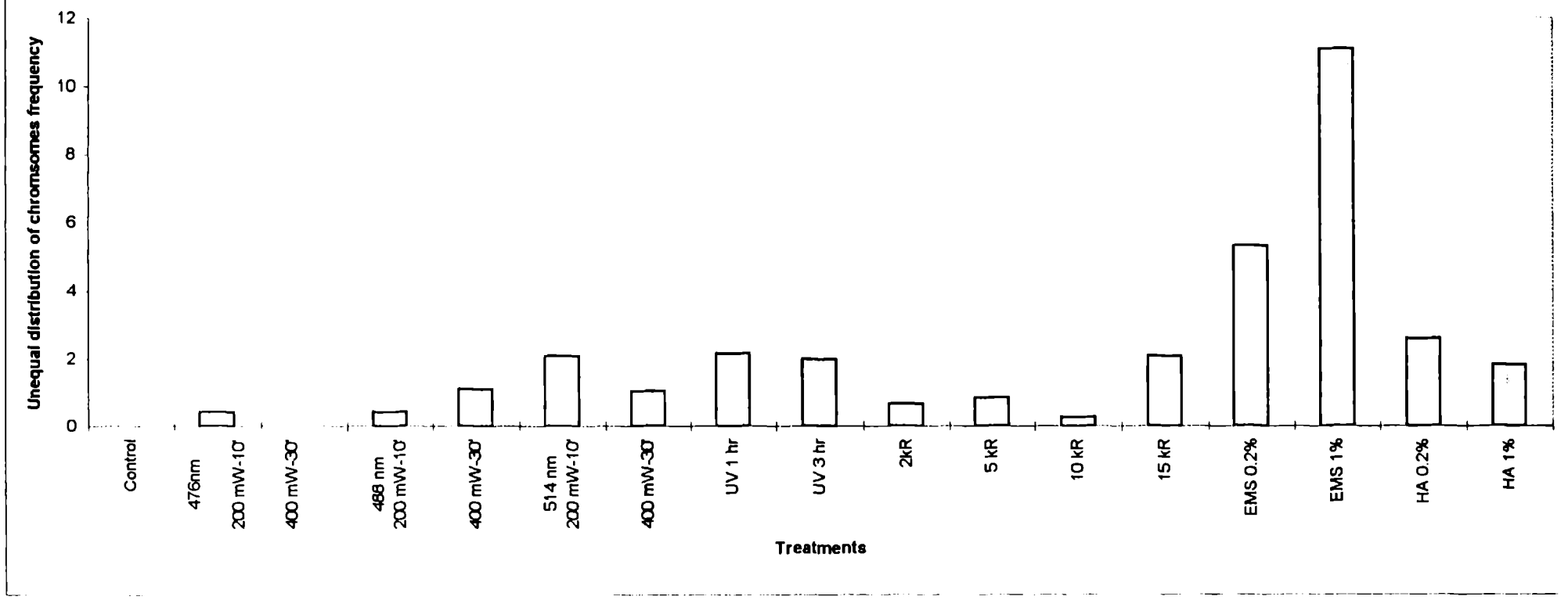
EMS induced laggards at 0.2% and HA at 1% (Fig.74B App.VIIIB). The frequency of this aberration induced by these two mutagens was lower than that induced by lasers.

4.3.5 Unequal distribution of chromosomes

This type of aberration was found at anaphase stage of meiosis in certain treatments(plate figs. 126,133). Unequal distribution of chromosomes was not observed in control and in exposures of 30 min at 400 mW of 476 nm laser. The aberration was most frequently observed in samples given 1% of EMS treatment.

In laser treatments at 476 nm, this aberration was found only in exposures of 10 min in power density 200 mW (Fig.75B App.VIIIB), but laser exposure at 488 nm induced dose dependent increase with increase in power density (Fig.75B App.VIIIB) while lasers of 514 nm induced dose dependent

Fig. 75 B : Effect of mutagens on unequal distribution of chromosomes in meiosis



decrease with increase in power density (Fig.75B App.VIIIB). The frequency of this aberration at 514 nm was higher than that induced by 476 and 488 nm.

UV exposure caused dose dependent decrease in unequal distribution of chromosomes with increase in exposure time (Fig.75B App.VIIIB). The frequency of this aberration induced by UV was slightly higher than that induced by lasers.

No dose dependence in the frequency of this aberration was observed in γ-irradiated samples (Fig.75B App.VIIIB) and the frequency was similar to that induced by lasers.

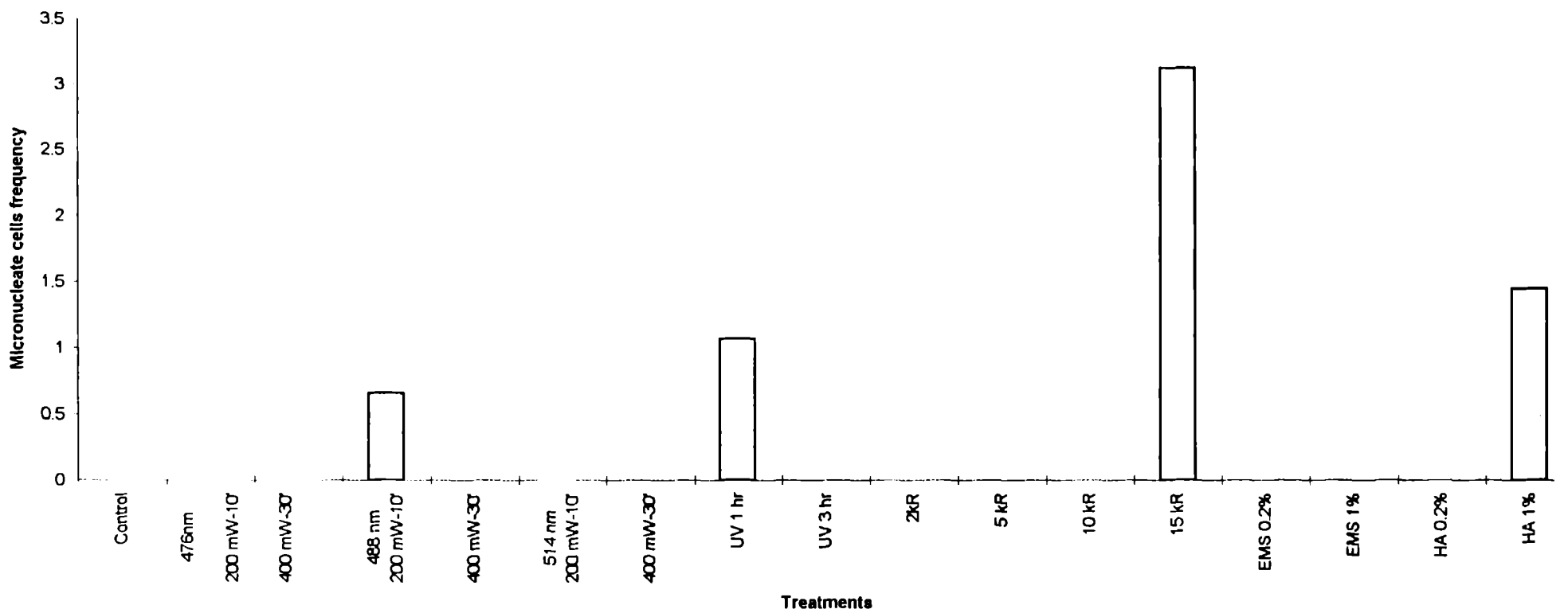
EMS treatment induced dose dependent increase in frequency of this aberration with increase in concentration (Fig.75B App.VIIIB). The frequency of this change induced by EMS was several fold higher than that induced by lasers.

HA treatments induced dose dependent decrease in unequal distribution of chromosomes with increase in treatment concentration (Fig.75B App.VIIIB). The frequency of this aberration induced by HA was higher than that induced by lasers.

4.3.6 Micronucleate Cells

Micronucleate cells were found mainly at telophase(plate figs 134, 135). Cells with micronuclei were found only in four mutagen treatments namely : 10 min exposure at 200 mW of 488 nm lasers, 1 hour of UV exposure, 15 kR of γ-irradiation and 1% of HA treatment. The frequency of this

Fig. 76 B : Effect of mutagens on micronucleate cells in meiosis



Figs. 117-136: Effect of mutagens on meiosis in *V. faba*

" 117 : Control-metaphase-1 with 6 bivalents.

" 118 : Pollen mother cell with clumping of chromosomes at prophase.

" 119 : Pollen mother cell with diakinesis ring.

" 120 : Pollen mother cell with clumping of chromosomes at diakinesis.

" 121 : Pollen mother cell with clumping of chromosomes at metaphase.

" 122 : Metaphase-1 with ring of 2 chromosomes and 5 bivalents.

" 123 : Anaphase-2 with bridge at 1 pole.

" 124 : Anaphase-2 with bridges.

" 125 : Late anaphase-2 with bridge at 1 pole.

" 126 : Anaphase-2 with unequal distribution of chromosomes and a chromosome ring at 1 pole.

" 127 : Anaphase-2 with tripolarity and chromosome bridges.

" 128 : Triad cell.

" 129 and 130: Telophase-1 with bridges.

" 131 and 132:Telophase-1 with laggards.

" 133 : Telophase-2 with unequal distribution of chromatin at poles.

" 134 : Telophase-1 with micronuclei.

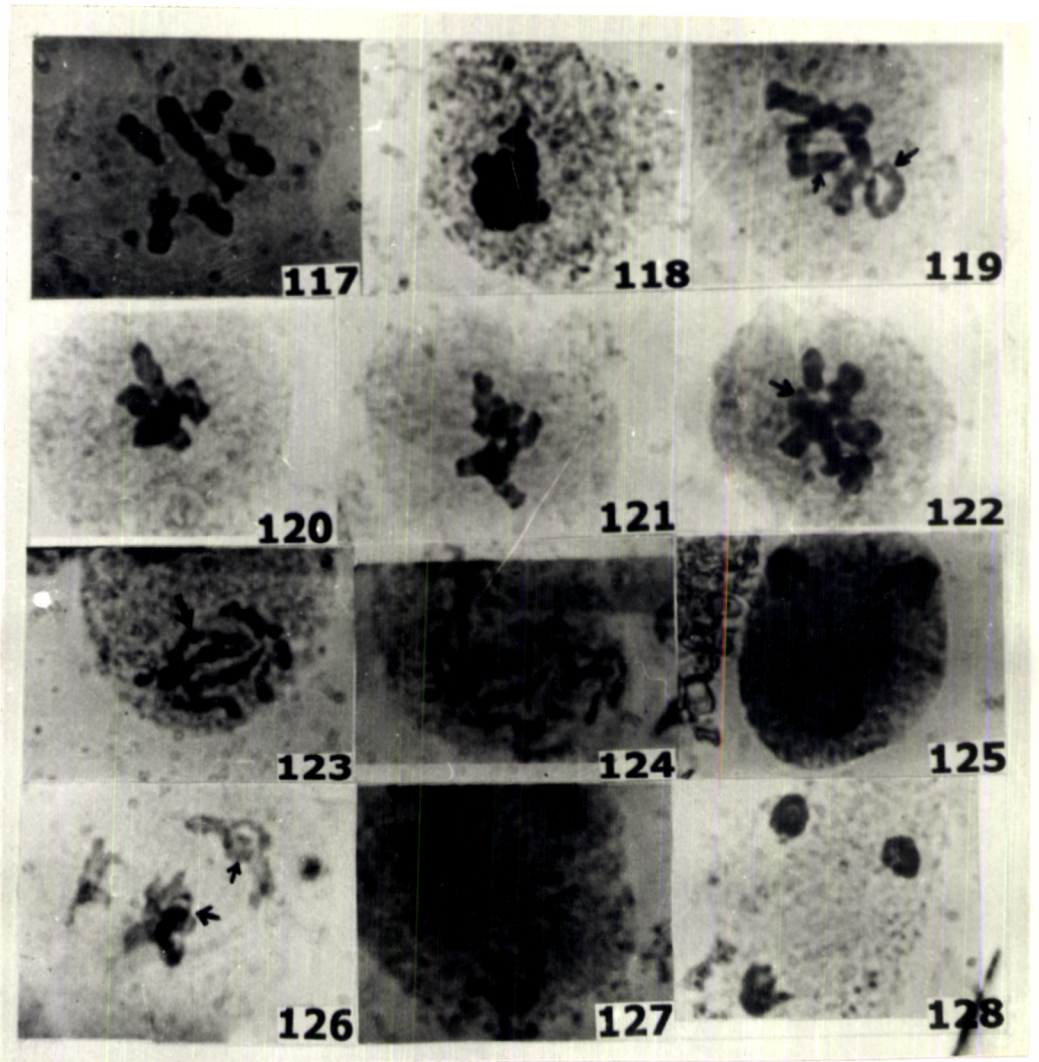
" 135 : Interphase pollen mother cell with micronuclei.

" 136 : Cytomixis. (X 1000)

" 137 : Fertile pollen grains. (X 100)

" 138 : Fertile pollen grains.

" 139 : Sterile pollen grains. (X 1000)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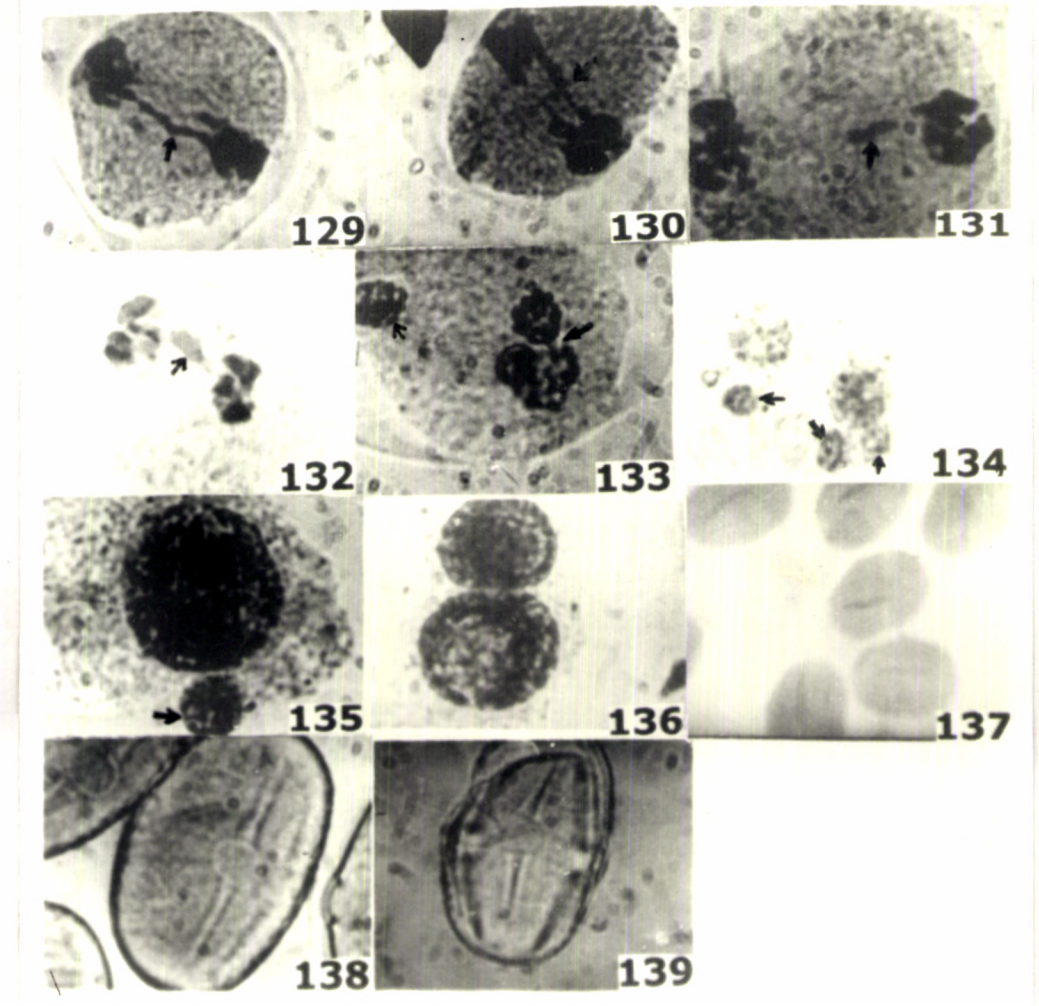
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parameter induced by 15 kR of γ-irradiation was higher than that induced by other treatments(Fig.76B App. VIIIB).

4.3.7 Total aberrations

The total aberration observed in control was low (0.6%).

Lasers induced dose dependent increase in total aberration with increase in power density in all the three wavelengths (Fig.77B App.VIIIB).

UV exposures induced dose dependent decrease in the total aberration with increase in exposure time (Fig.77 App. VIIIB). The frequency of this parameter caused by UV was similar to that induced by laser exposures.

γ-irradiations induced dose dependent increase in total aberration with increase in radiation dose (Fig.77B App.VIIIB). Total aberration induced by γ-irradiations were higher than that induced by lasers.

EMS treatment also induced dose dependent increase in this parameter with increase in concentration (Fig.77B App.VIIIB) The total aberration induced by EMS treatment was higher than that induced by laser exposures and 1% EMS treatment induced aberration several fold higher than that induced by lasers.

HA treatments induced dose dependent decrease in total aberration (Fig.77B App.VIIIB), although the values were similar to that induced by lasers.

4.4 Pollen sterility

Control (untreated) samples showed the lowest and EMS treatment at 1% level showed the highest percentage of sterile pollen grains.

Fig. 77 B : Effect of mutagens on total meiotic aberrations

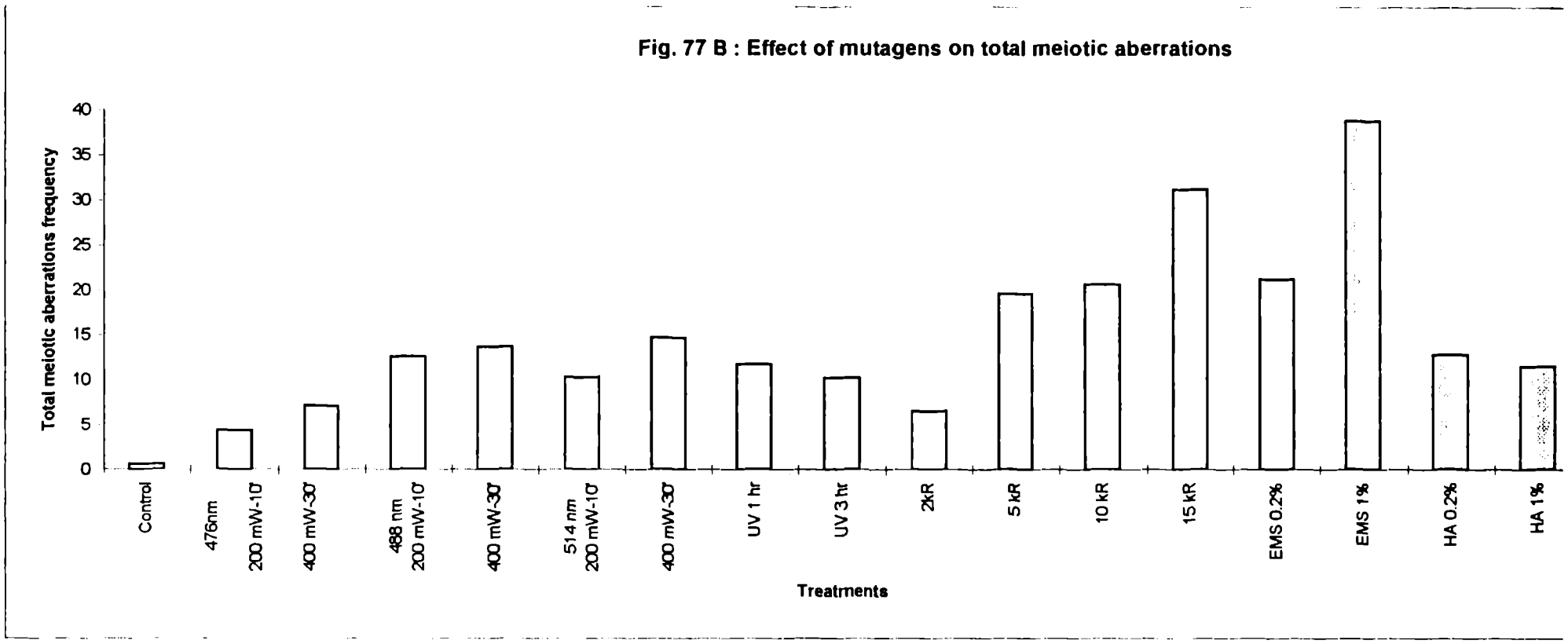
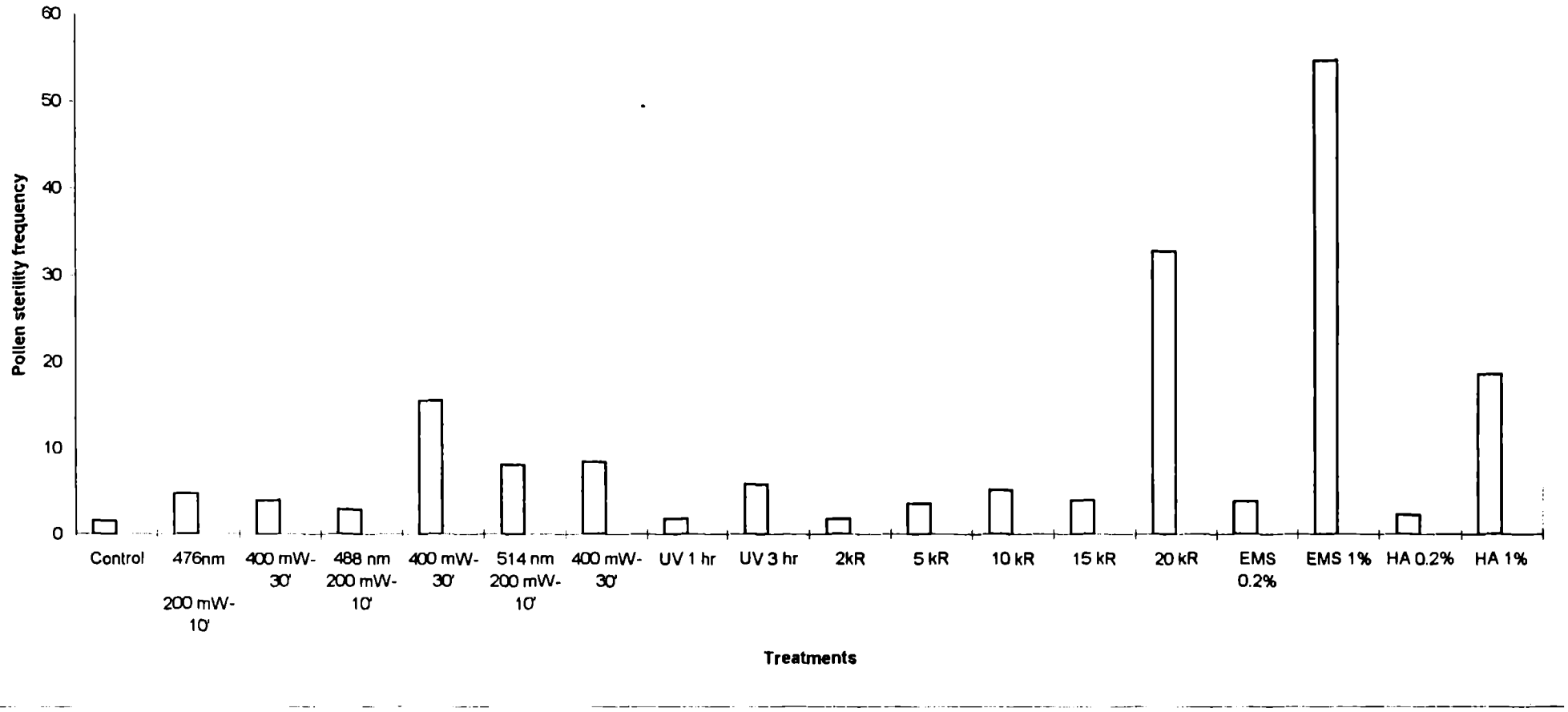


Fig. 78 B : Effect of mutagens on pollen sterility in V. faba



The pollen sterility induced by EMS 1% treatment was several fold higher to that caused by other treatments.

In laser exposures, a dose dependent increase in pollen sterility with increase in treatment dose at 488 and 514 nm (Fig.78B App.VIIIB) was observed while laser exposure at 476 nm induced decrease in pollen sterility (Fig.78B App.VIIIB). Among the different laser treatments, the frequency of this parameter induced by exposures of 30 min in power density 400 mW at 488 nm was highest and that of 10 min in power density 200 mW of 488 nm was lowest. The frequency of pollen sterility induced by 514 nm was higher than that induced by 476 nm.

UV also induced dose dependent increase in pollen sterility with increase in power density (Fig.78B App.VIIIB). In UV exposures of 1 hour, pollen sterility frequency was lower than that induced by laser exposures while in UV exposure of 3 hours, frequency of this parameter was similar to that induced by 476 and in exposures of 10 min at power density 200 mW of 488 and lower to 514 nm and exposures of 30 min at power density 400 mW of 488 nm.

γ-irradtions induced dose dependent increase in pollen sterility with increase in irradiation dose (Fig.78B App.VIIIB). The frequency of this parameter at 20 kR was several fold higher than that induced by other doses of γ-irradiations and by laser exposure. The frequency of pollen sterility induced by 2 kR was lower than that induced by lasers.

EMS caused dose dependent increase in pollen sterility with increase in concentration (Fig.78B App.VIIIB). The frequency of this parameter induced by 1% EMS concentration was several fold higher than that induced by

laser exposures while 0.2% of EMS treatment induced pollen sterility in frequencies similar to that induced by lasers at 476 nm and exposures of 10 min at power density 200 mW of 488 nm and lower than that induced by 514 nm and by exposures of 30 min at power density 400 mW of 488 nm.

Pollen sterility increased with increase in HA concentration (Fig. 78BApp.VIIIB). The frequency of this parameter induced by HA at 0.2% was lower and at 1% level , it was higher than that induced by laser exposures.

4.5 Germination/sprouting index, growth and yield studies

Seeds of *V. faba* and bulbs of *A. cepa* subjected to selected treatments of mutagens (as detailed in materials and methods) were grown in the field to study the effect of the mutagens on germination/sprouting, growth and yield. The experiment was repeated twice with quantitatively similar results, one of which is reported here. Gamma irradiations of *A. cepa* bulbs inhibited the sprouting of bulbs even at a dose of 2kR. The bulbs produced leaves and roots of a few millimetre length during the initial stage of sprouting. Further sprouting of bulbs ceased in all γ-irradiated *A. cepa* bulbs. Due to this it was not possible to carry out further studies in *A. cepa*.

4.5.1 Germination/sprouting index

In *A. cepa,* laser exposure at 476 nm induced no dose dependent change in sprouting index with increase in power density (Fig.61A App.VIIA) and the index was similar to that of control (100%). Laser exposures of 488 nm induced dose dependent increase in sprouting index with increase in power density (Fig.61A App.VIIA) and the index induced by exposures of 10 min in power density 200 mW was lower to control and by 30 min in power density

400 mW was similar to control. Laser exposure at 514 nm caused dose dependent decrease in the sprouting index with increase in power density (Fig. 61A App.VIIA). The index induced by exposures of 10 min in power density 200 mW was higher and by 30 min power density 400 mW was similar to control. Sprouting index induced by laser exposures at 476 nm was higher than that induced by 488 nm and exposures of 30 min in power density of 514 nm and similar to that induced by exposures of 10 min in power density of 200 mW of 514 nm. In *V. faba,* lasers of 476 nm induced dose dependent increase in germination index with increase in power density (Fig.79B App.IXB) and the index was lower than that of control. Laser exposures at 488 nm induced dose dependent increase in germination index (Fig.79B App.IXB). The index observed in exposures of 10 min in power density 200 mW was lower and 30 min in power density 200 mW was higher than that of control. 514 nm induced dose dependent decrease in germination index with increase in power density (Fig.79B App.IXB) and was lower than that of control. Germination index frequency induced by lasers of 476 nm was lower than that induced by lasers of 488 and 514 nm. Germination index induced by exposures of 10 min in power density 200 mW of 488 and 514 nm were similar and that induced by 30 min in power density 400 mW of 488 nm was higher than that induced by 514 nm lasers.

In *A. cepa* UV exposures induced dose dependent increase in sprouting index with increase in exposure time (Fig.61A App.VIIA) and the index was higher than that of control. Sprouting index induced by UV exposures of 1 hour was lower than that induced by laser exposures at 476 nm and by exposures of 10 min in power density 200 mW of 514 nm, similar to 30 min in power density 400 mW of 488 and 514 nm and higher than 10 min in power

density 200 mW of 488 nm. UV exposures in *V. faba* induced dose dependent decrease in germination index (Fig.79B App.IXB) and the index was lower than that of control. The germination index induced by UV was higher than that induced by laser exposure at 476 nm.

In *V. faba,* γ-irradiations induced dose dependent decrease in germination index with increase in irradiation dose (Fig.79B App.IXB). The index observed in samples irradiated with 15 and 20 kR was several fold lower than that with 2,5 and 10 kR. The germination index in control (untreated) samples and that in 2 and 5 kR samples was similar.

In *A. cepa,* EMS treatment induced dose dependent increase in sprouting index with increase in treatment concentration (Fig.61A App.VIIA). The index in EMS treated samples was higher than that of control. Sprouting index in *A. cepa* induced by EMS (0.2%) was lower than that induced by laser exposures in all three wavelengths. The same induced by EMS (1%) was similar to that induced by 476 nm lasers. In *V. faba,* EMS caused dose dependent decrease in germination index with increase in EMS concentration (Fig.79B App.IXB). The index in samples treated with 0.2% EMS was higher and in 1%, it was similar to that of control (untreated) samples. The germination index induced by 0.2% EMS treatment in *V. faba* was higher than that induced by laser exposures. The index caused by 1% EMS treatment was similar to that induced by exposures of 30 min in power density 400 mW of 488 nm and higher than that induced by other laser exposures.

In *A. cepa,* HA induced dose dependent decrease in sprouting index with increase in concentration (Fig.61A App.VIIA). The index in samples treated with 0.2% of HA was similar and with 1% HA was several fold lower

Fig. 61 A : Effect of mutagens on sprouting index in *A. cepa*

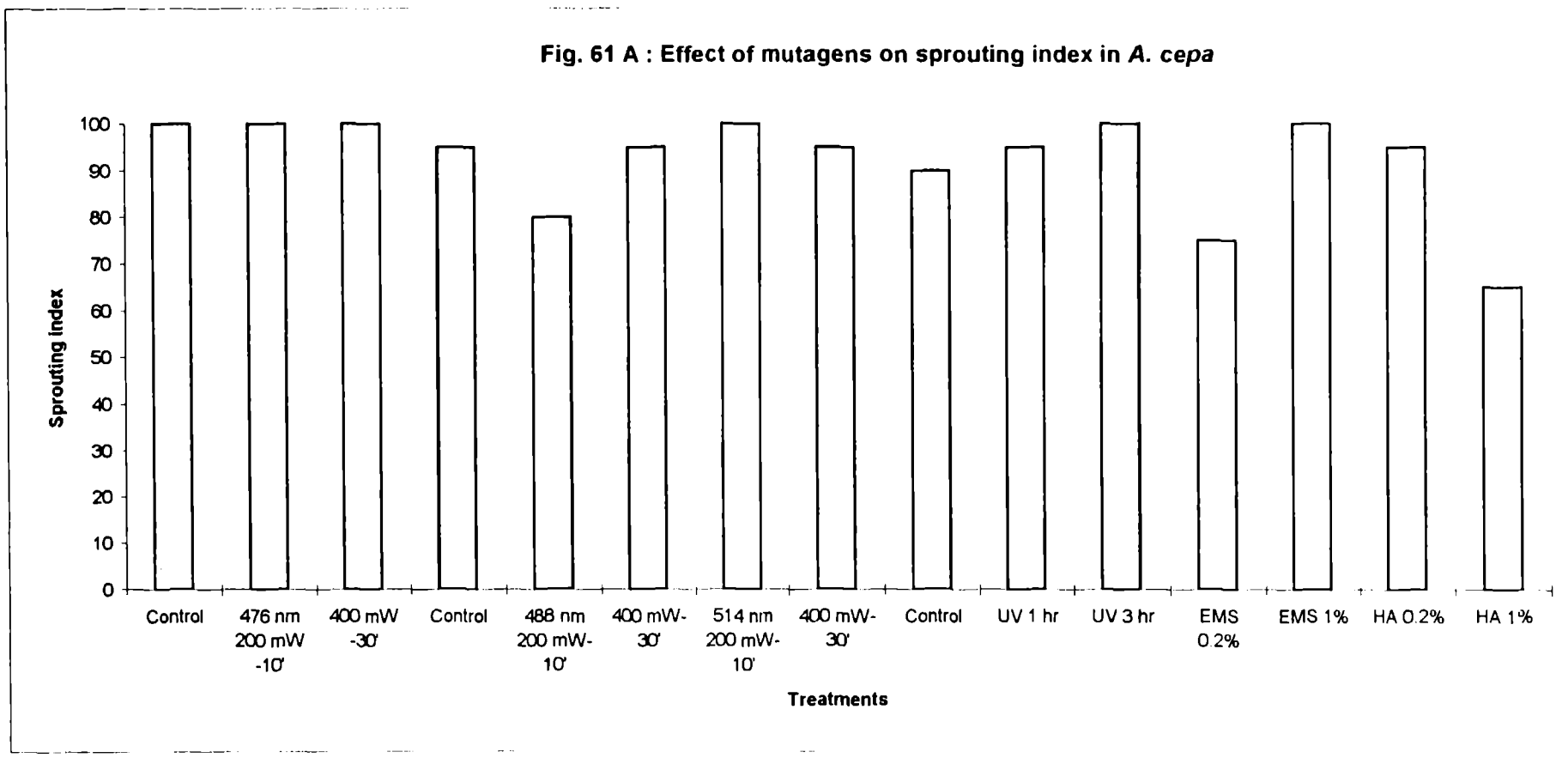
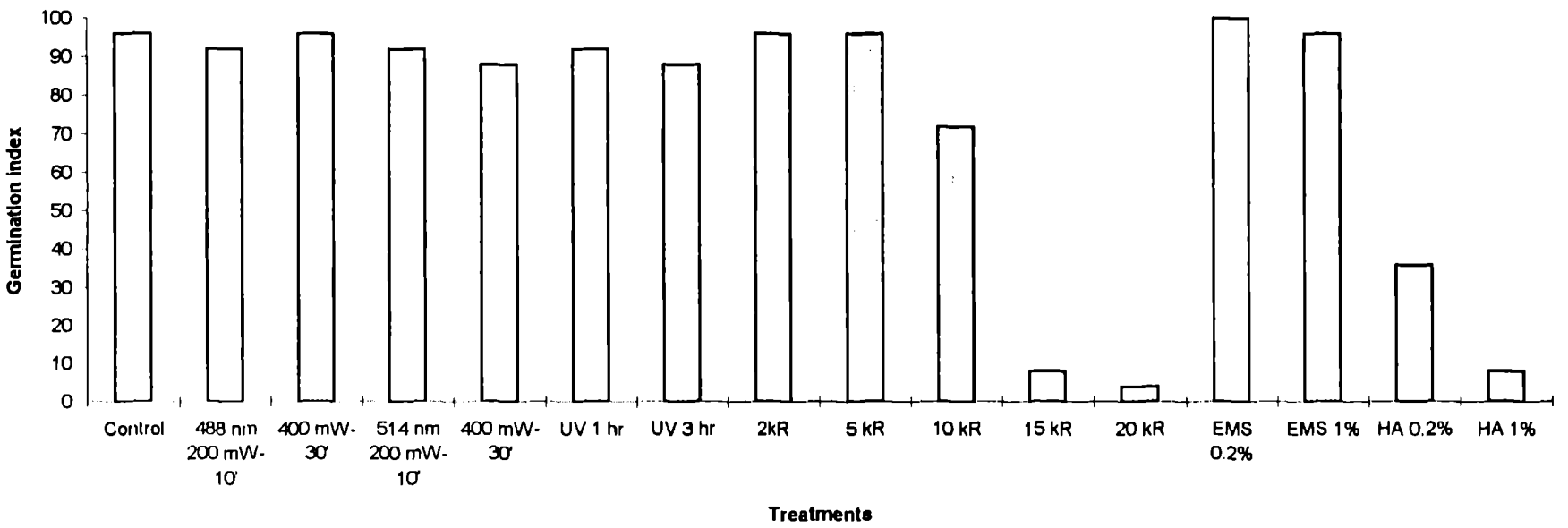


Fig. 79 B : Effect of mutagens on germination index in *V. faba*



than that of control samples. The sprouting index caused by 0.2% HA treatment was lower than that induced by laser exposure at 476 nm. In *V. faba* also, HA induced dose dependent decrease in germination index with increase in HA concentration (Fig.79B App.IXB) and the index was several fold lower than that of control (untreated) samples.

Comparing the sprouting index in *A. cepa* with the germination index of *V. faba*, laser exposures of 476 nm induced no change in sprouting index in *A. cepa* but 488 nm induced increase in sprouting index and 514 nm decrease in sprouting index with increase in power density. However in *V. faba* laser exposures of 476 nm and 488 nm induced increase in germination index but 514 nm caused decrease in germination index with increase in power density was found. UV exposures induced increase in sprouting index in *A. cepa* but decrease in germination index in *V. faba* with increase in exposure time was observed. EMS treatment caused increase in sprouting index in *A. cepa* but decrease in germination index in *V. faba* with increase in treatment concentration. HA induced decrease in sprouting/germination index with increase in treatment concentration in both plant species.

4.5.2 Growth

The change in plant height over a period of time or the growth rate of mutagen treated samples was compared with that of the control (untreated) samples (plate figs. 1A to 12A and 1B to 14 B).

In *A. cepa,* growth rate induced by all three wavelengths of laser exposures was higher than control (Fig.62A, 63 A, 64A App.VIIA). Growth rate induced by 514 nm was higher than 488 nm. Increase in growth rate with increase in power density was observed at 488 nm and 514 nm but decrease in

growth rate with increase in power density was found at 476 nm. In *V. faba,* growth rate induced by laser exposure of 476 nm was similar to control (Fig.80B App.IXB) but the growth rate induced by 488 nm and 514 nm laser exposures was higher than control (Figs. 81B and 82B App.IXB). Growth rate induced by 488 nm showed increase with increase in power density but laser exposures of 476 nm and 514 nm induced decrease in plant growth with increase in power density.

In *A. cepa,* UV exposures caused growth rate similar to control (Fig.65A App.VIIA) but in *V. faba* UV exposures induced growth rate higher than control (Fig.83B App.IXB). Growth rate of 3 hour exposure to UV was lower to 1 hour exposure in both plant species. Growth rate induced by UV exposures was slightly lower than that induced by 488 nm and 514 nm laser exposures.

γ-irradiation in *V. faba* induced dose dependent decrease in growth rate with increase in irradiation dose (Fig.84B App.IXB). The growth rate in 2 and 5 kR γ - irradiated samples was similar to control and in 10 kR the growth rate was lower than control. Growth rate induced by γ-irradiation was lower than the growth rate induced by 488 and 514 nm of laser exposures.

EMS treatment induced growth rate lower than control in *A. cepa* (Fig.66A App.VIIA) but in *V. faba* EMS induced growth rate higher than control (Fig.85B App.IXB). Growth rate caused by 0.2% of EMS treatment was higher than that caused by 1 % EMS treatment in both plant species. Growth rate induced by EMS treatment was lower than that induced by 488 nm and 514 nm of laser exposures.

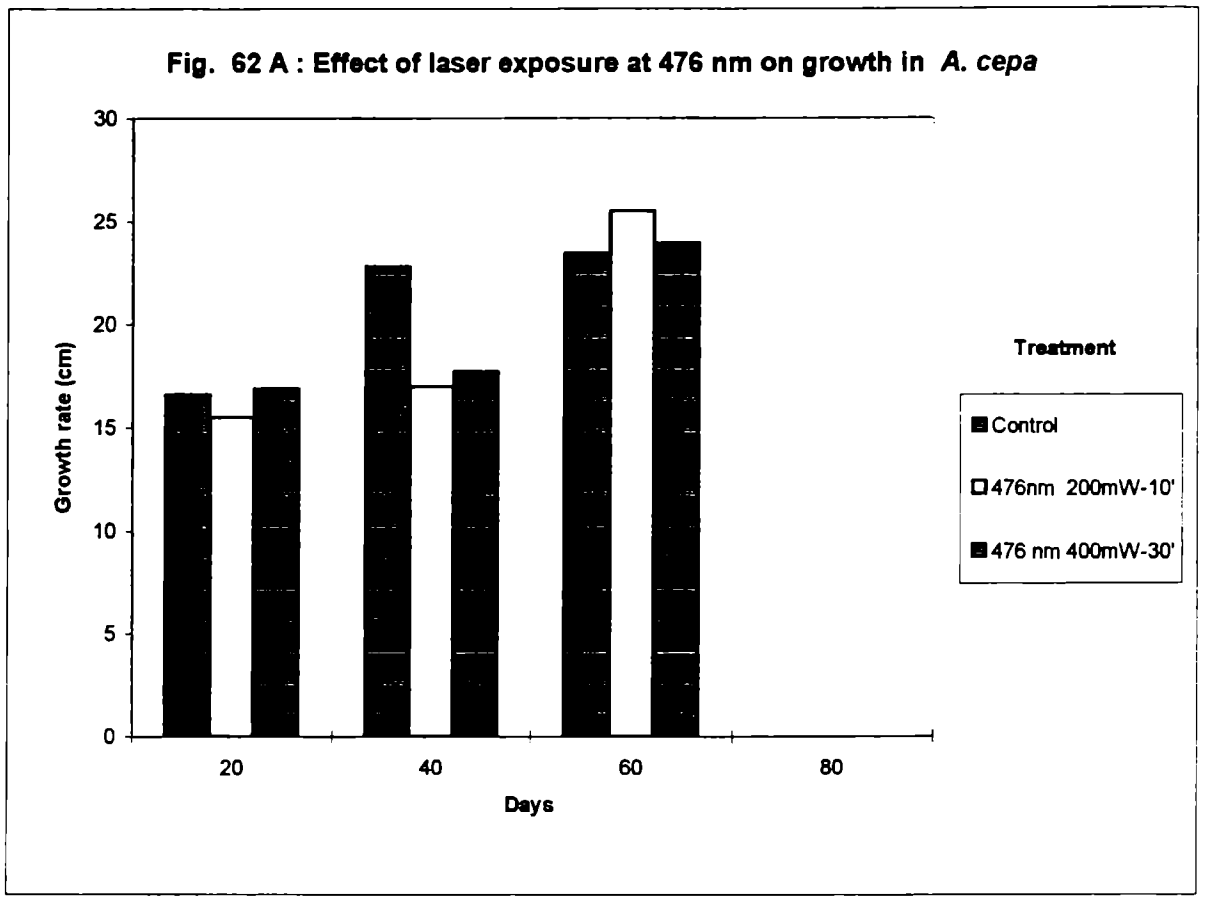


Fig. 62 A : Effect of laser exposure at 476 nm on growth in A. cepa
Growth rate (cm)
30
25
20
15
10
5
0
20
40
60
80
Days
Treatment
Control
476nm 200mW-10'
476 nm 400mW-30'

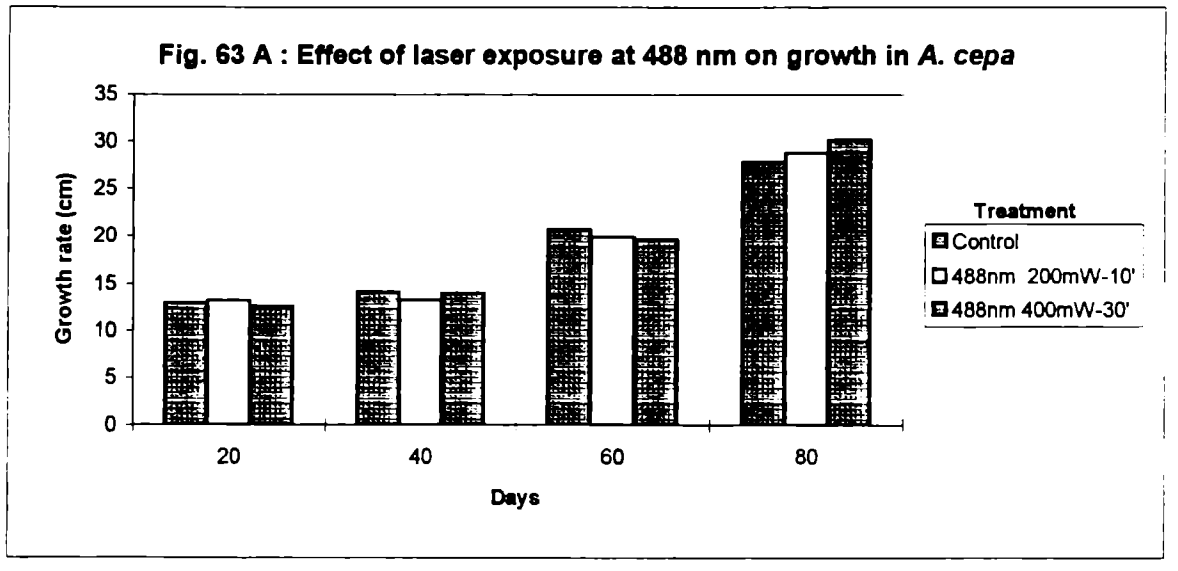


Fig. 63 A : Effect of laser exposure at 488 nm on growth in A. cepa
Growth rate (cm)
35
30
25
20
15
10
5
0
20
40
60
80
Days
Treatment
Control
488nm 200mW-10'
488nm 400mW-30'

Fig. 64 A : Effect of laser exposure at 514 nm on growth in *A. cepa*

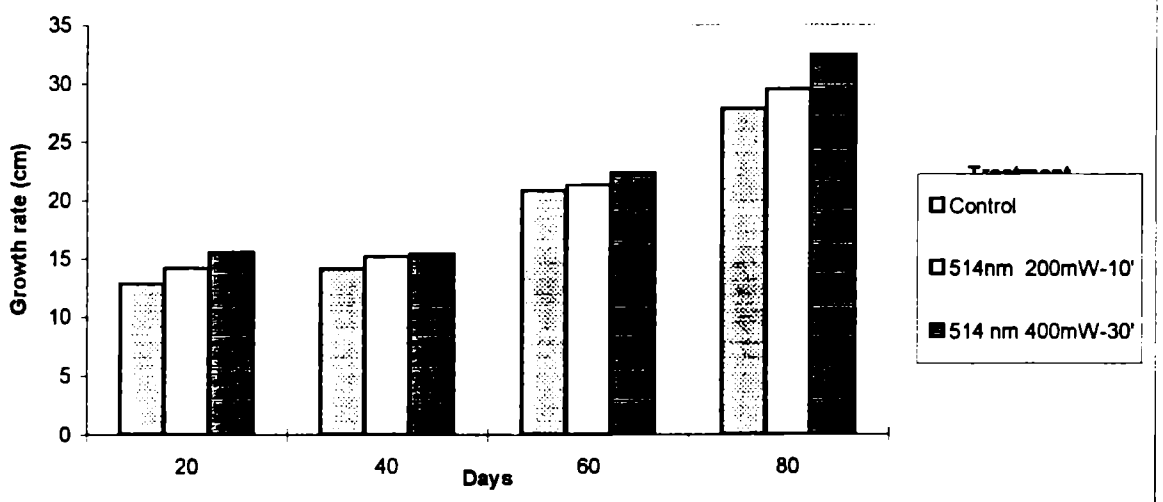


Fig. 65 A : Effect of UV exposure on growth in *A. cepa*

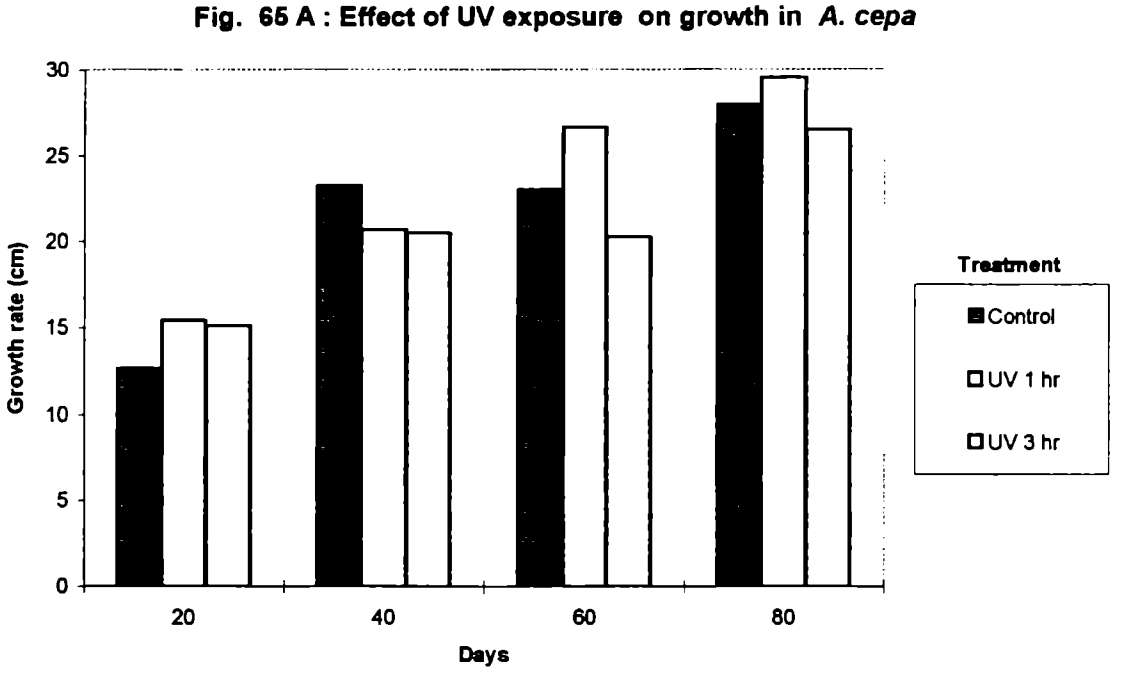


Fig. 66 A : Effect of EMS treatment on growth in *A. cepa*

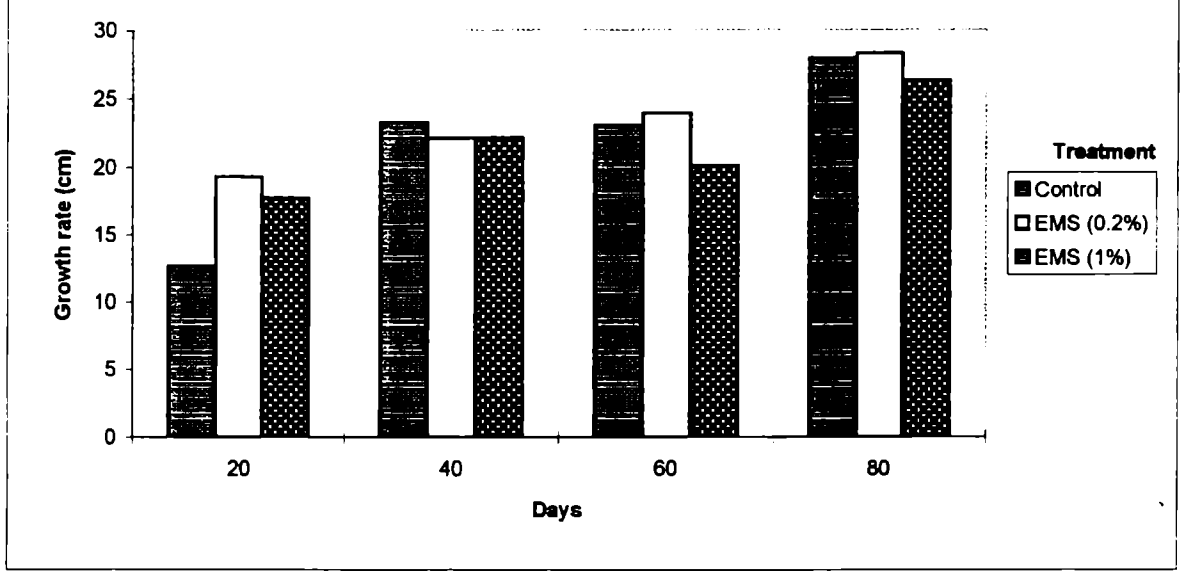


Fig. 67 A : Effect of HA treatment on growth in *A. faba*

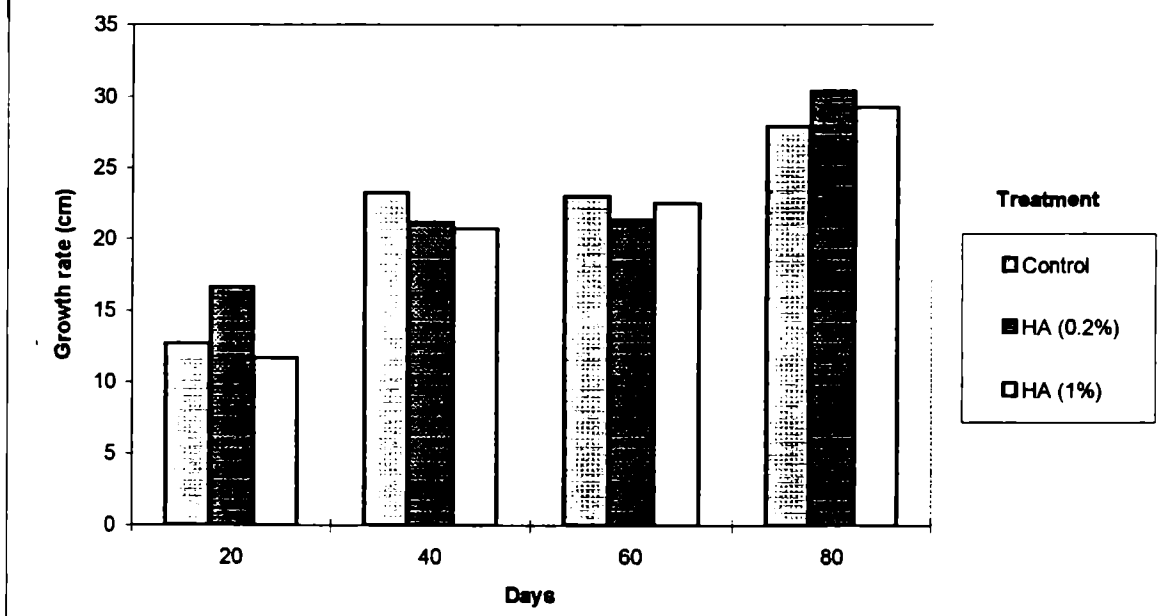


Fig. 80 B : Effect of laser exposure at 476 nm on growth in *V. faba*

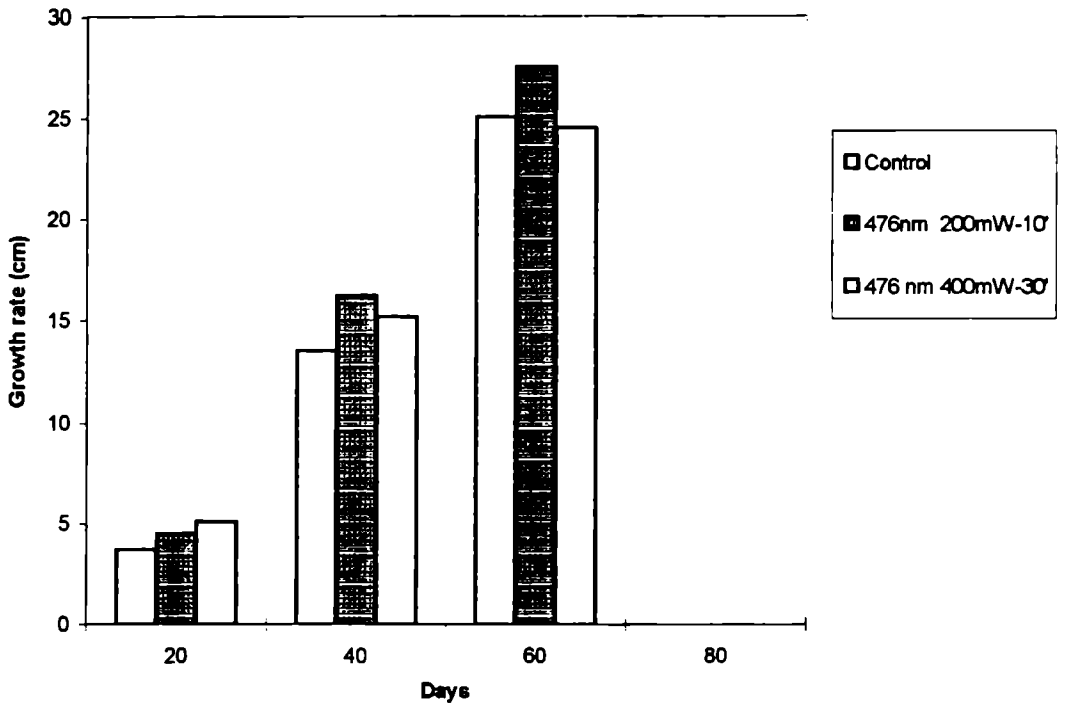


Fig. 81 B : Effect of laser exposure at 488 nm on growth in *V. faba*

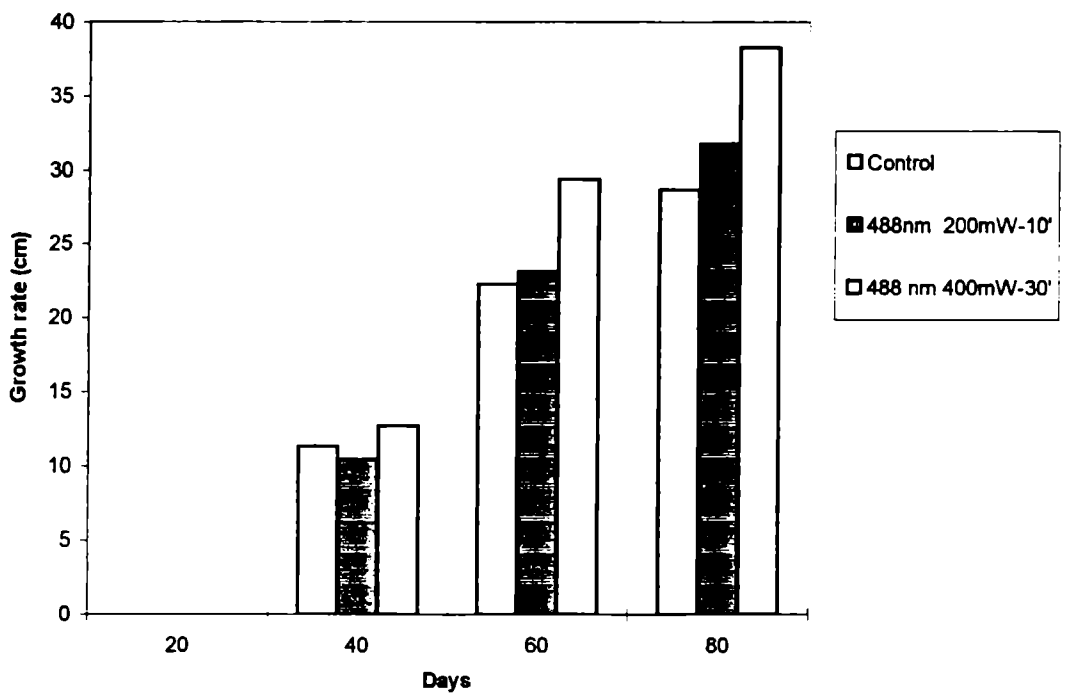


Fig. 82 B : Effect of laser exposure at 488 nm on growth in *V. faba*

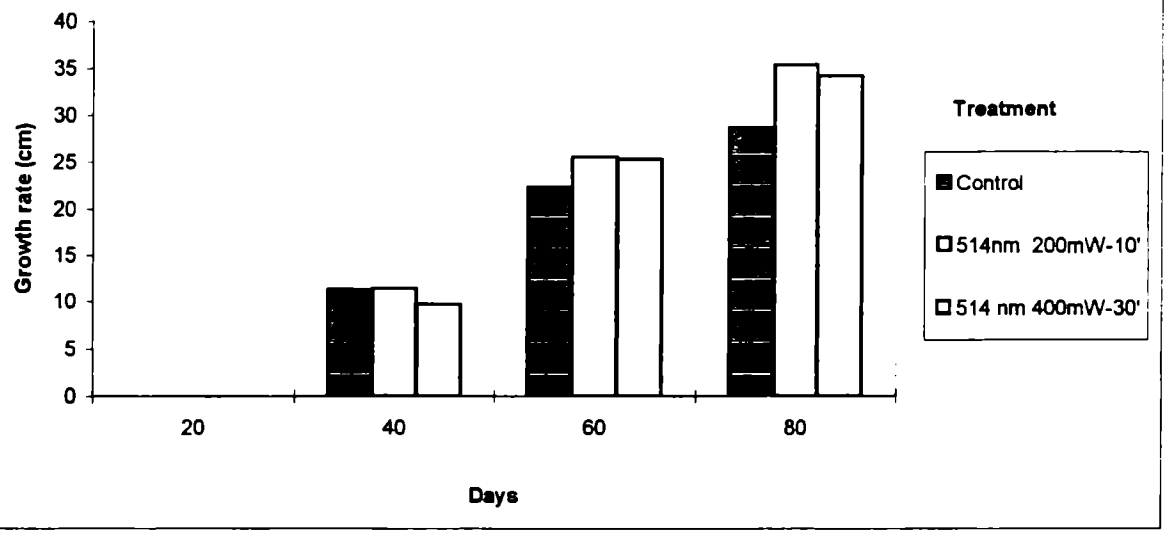


Fig. 83 B : Effect of UV exposure on growth in *V. faba*

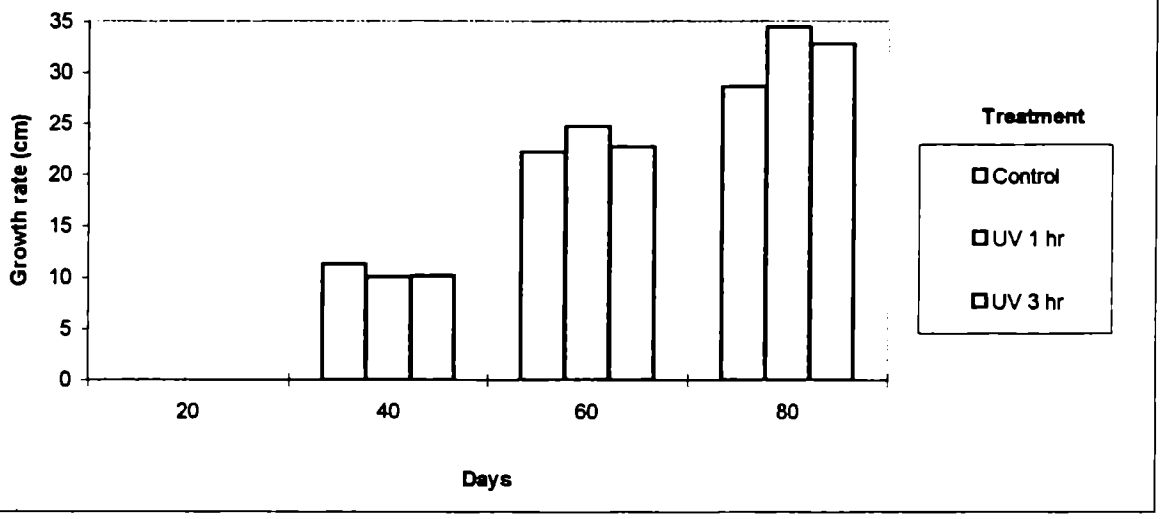


Fig. 84 B : Effect of gamma irradiation on growth in *V. faba*

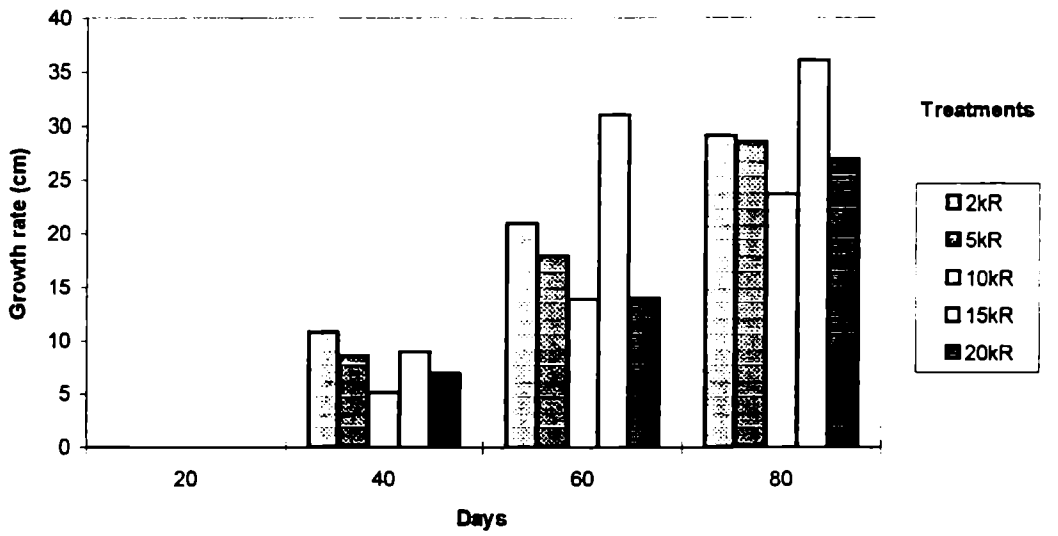
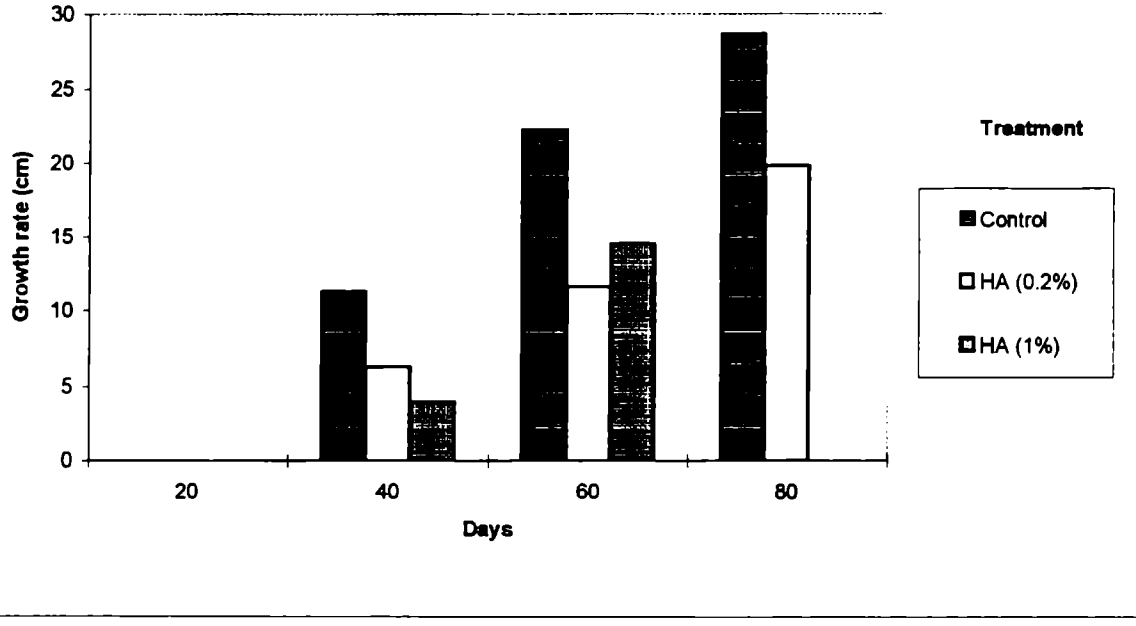


Fig. 85 B : Effect of EMS treatment on growth in *V. faba*

Fig. 86 B : Effect of HA treatment on growth in *V. faba*



Photographs 1A-12A - Effect of mutagens on growth in *Allium cepa*

1A. Control

2A. Effect of laser exposure at 488 nm at 200mW on growth

3A. Effect of laser exposure at 488 nm at 400mW on growth

4A. Effect of laser exposure at 514 nm at 200mW on growth


1A


2A


3A


4A

5A. Effect of laser exposure at 514 nm at 400mW on growth

6A. Control

7A. Effect of UV exposure at 1 hr on growth

8A. Effect of UV exposure at 3 hr on growth


5A


6A


7A


8A

9A. Effect of EMS treatment at 0.2% on growth

10A. Effect of EMS treatment at 1% on growth

11A. Effect of HA treatment at 0.2% on growth

12A. Effect of HA treatment at 1% on growth


9A


10A


11A


12A

Photographs 1B - 14B - Effect of mutagens on growth in *Vicia faba* :

1B. Control

2B. Effect of gamma irradiation at 2kR on growth

3B. Effect of gamma irradiation at 5 kR on growth

4B. Effect of gamma irradiation at 10 kR on growth


1B


2B


3B


4B

5B. Effect of gamma irradiation at 15 kR on growth

6B. Effect of gamma irradiation at 20 kR on growth

7B. Effect of laser exposure at 488 nm at 200mW on growth

8B. Effect of laser exposure at 488 nm at 400mW on growth


5B


6B


7B


8B

9B. Effect of laser exposure at 514 nm at 200mW on growth

10B. Effect of laser exposure at 514 nm at 400mW on growth

11B. Effect of UV exposure at 1 hr on growth

12B. Effect of UV exposure at 3 hr on growth


9B


10B


11B


12B

13B. Effect of EMS treatment at 0.2% on growth

14B. Effect of EMS treatment at 1% on growth


13B


14B

HA treatment caused growth rate higher than control in *A. cepa* (Fig.67A App.VIIA). Growth rate in HA treatments was similar to that induced by 488 nm and 514 nm lasers but in *V. faba* HA treatment induced growth rate lower to control and that induced by laser exposures of 488 and 514 nm (Fig. 86B App. IXB).

Comparing the growth rate in *A. cepa* and *V. faba*, laser exposure of 476 nm, induced growth rate lower than control but 488 nm and 514 nm induced growth rate higher than control in *A. cepa* while in *V. faba* growth rate induced by 476 nm laser exposure was similar to control but 488 nm and 514 nm of laser exposures induced growth rate higher than control. UV exposures induced growth rate similar to control in *A. cepa* but in *V. faba* UV induced growth rate higher than control. EMS treatment induced growth rate lower than control in *A. cepa* but in *V. faba* EMS induced growth rate higher than control. In *A. cepa,* HA treatment induced growth rate higher than control but in *V. faba* plants treated with 1% HA did not attain full growth and the growth rate of HA 0.2% treatment was lower than control.

4.5.3 Yield

Yield was computed as the total seed weight (or bulb weight) divided by the total number of plants in each treatment. The yield in each treatment was compared with that of control. Growth of the plants in 476 nm of laser exposure before attaining final stages of plant growth were damaged by heavy rains. Because of this the seeds/bulbs were not fully developed for yield analysis. Maximum yield in both plant species was found in samples exposured for 30 min at power density 400 mW of 488 nm.

In *A. cepa,* laser exposures at 488 and 514 nm induced dose dependent increase in yield with increase in power density (Fig.68A App.VIIA). The yield induced by laser exposures of 10 min in power density 200 mW of 488 nm was similar to and that induced by other laser exposures was almost double the yield of control. Laser exposures in *V. faba* induced dose dependent increase in yield with increase in power density at 488 nm (Fig.87B App.IXB) but at 514 nm dose dependent decrease in yield with increase in power density was observed (Fig.87B App.IXB). The yield induced by laser exposures at 488 nm was higher than that of control and was found highest in exposures of 30 min in power density 400 mW of 488 nm. 514 nm laser exposure induced yield values lower than control in exposures of 30 min in power density of 400 mW and higher than control in exposures of 10 min in power density 200 mW.

In *A. cepa,* UV induced a dose dependent decrease in yield with increase in exposure time (Fig.68A App.VIIA). The yield induced by UV exposures for 1 hour was higher and 3 hour was lower than that of control. In *V. faba* also, UV induced dose dependent decrease in yield with increase in exposure time (Fig.87B App.IXB). Yield induced by UV exposure of 1 hour was higher and of 3 hour was similar to the control value.

γ-irradiation in *V. faba* also induced dose dependent decrease in yield with increase in irradiation dose (Fig.87B App.IXB). The yield in γ - irradiated samples was lower than that of control.

In *A. cepa,* EMS treatment induced dose dependent decrease in yield with increase in treatment dose (Fig.68A App.VIIA). The yield induced by EMS treatment was higher than that of control value. In *V. faba* also, EMS induced dose dependent decrease in yield with increase in treatment

Fig. 68 A : Effect of mutagens on yield in *A. cepa*

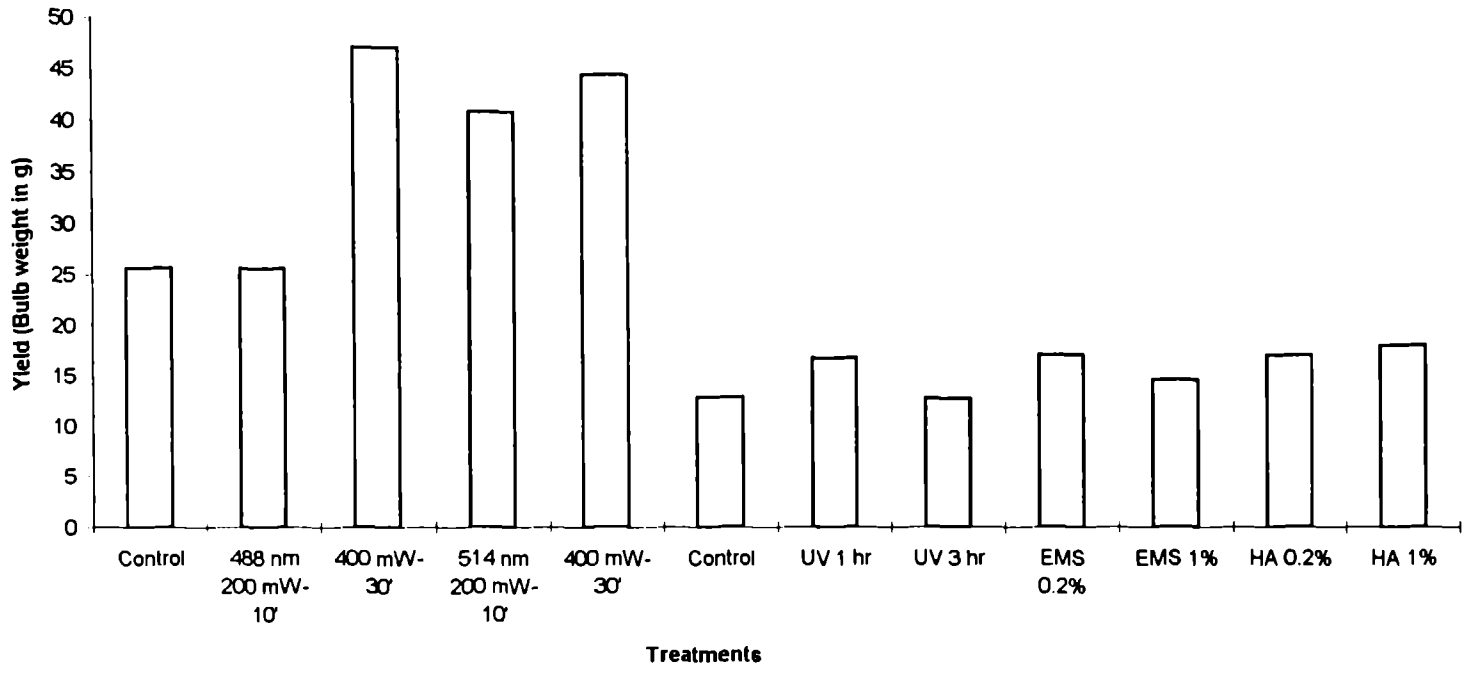
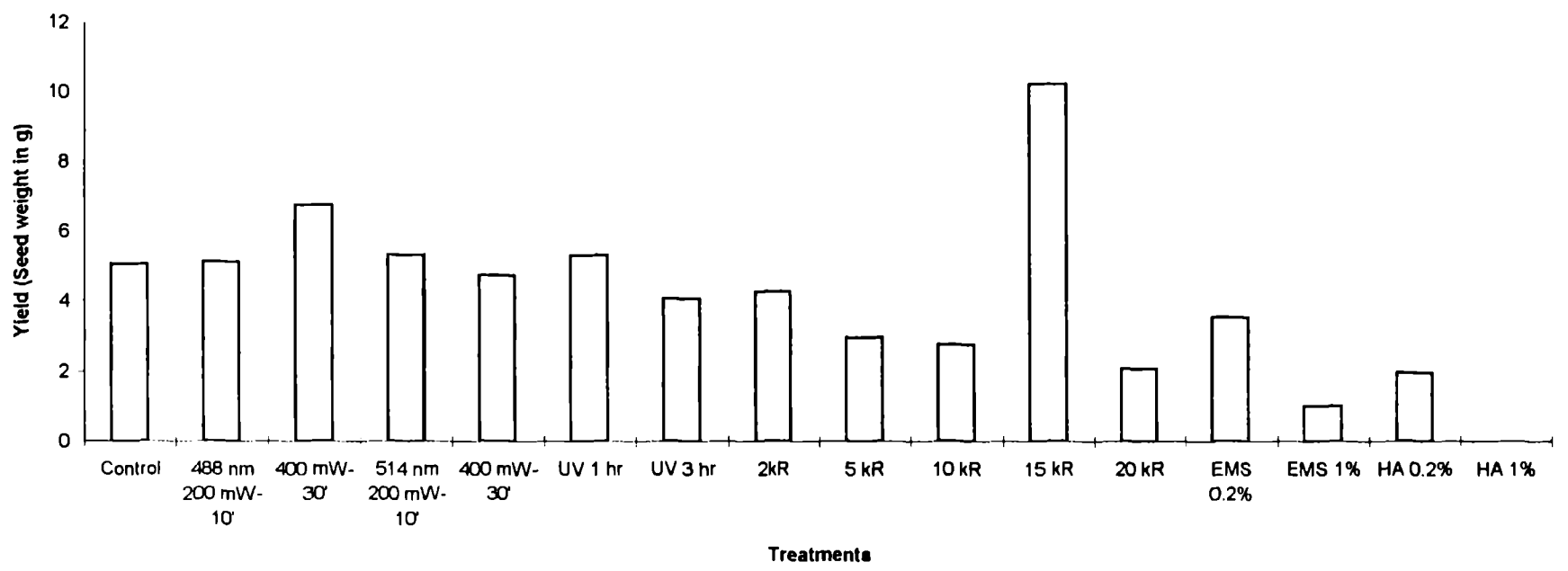


Fig. 87 B : Effect of mutagens on yield in *V. faba*



concentration (Fig.87B App.IXB). The yield induced by 0.2% EMS treatment was lower than that of control value but in 1% EMS treated samples yield was found to be several fold lower than that of control value.

HA treatment in *A. cepa* induced dose dependent increase in yield with increase in treatment concentration (Fig.68A App.VIIA). The yield induced by HA was higher than that of control. But in *V. faba*, plants treated with 1% HA did not survive in last phase of growth. The yield induced by 0.2% of HA treatment was several fold lower than that of control (Fig.87B App.IXB)

In both *A. cepa* and *V. faba,* lower exposures of 488 nm induced increase in yield with increase in power density. Lasers of 514 nm caused a dose dependent increase in yield in *A. cepa* but a decrease in *V. faba*.

UV exposures induced dose dependent decrease in yield in both plant species. EMS caused dose dependent decrease in yield in both plant species. HA caused dose dependent increase in yield with increase in treatment concentration in *A. cepa* but in *V. faba* HA induced complete damage of the plants at 1% concentration before attaining full growth.

4.6 Effects of laser, gamma and ems on soluble protein content and enzyme activity

4.6.1 Effect on soluble protein content

In *A. cepa,* laser exposures of 476, 488 and 514 nm induced increase in soluble protein content in inner true leaves and in outer fleshy scale leaves higher than control. Soluble protein content was higher in outer fleshy scale leaves than in inner true leaves. Soluble protein content on fourth day was higher than on second day (Fig.69A App.VIIIA). In *V. faba*, laser exposures of 476 nm and 488 nm induced increase in soluble protein content in

cotyledon but 514 nm induced decrease in soluble protein content in cotyledon when compared to control. The soluble protein content in embryo, was less than control value on third day but on sixth day it was similar to control value in all three wavelengths of laser exposures. The soluble protein content in cotyledon was higher than that of the embryo in control, 476 and 488 nm of laser exposures but at 514 nm of laser exposures soluble protein content in embryo was higher than that in cotyledon (Fig.88B App.XB).

In *A. cepa,* γ-irradiation induced dose dependent decrease in soluble protein content in inner true leaves on second day and it was lower than control value. In outer fleshy scale leaves also soluble protein content in γ-irradiated samples was lower than control on second day and it was not dose dependent. Soluble protein content in inner true leaves was higher than in outer fleshy scale leaves (Fig.70A App.VIIIA). In *V. faba* γ-irradiation induced considerable variation in protein content on third day and sixth day in samples treated with different doses. Sixth day soluble protein content was higher in cotyledon in all γ-irradiated doses than control and maximum in samples irradiated with 10 kR. But on third day soluble protein content in cotyledon of γ-irradiated samples were similar to control. In embryo soluble protein content on third day in γ-irradiated samples was less than control but on sixth day soluble protein content in embryo of γ-irradiated samples were similar to control value. Total soluble protein content was higher in cotyledon than in embryo (Fig.89B App.XB).

In *A. cepa,* EMS treatment resulted in decrease in soluble protein content in inner true leaves and in outer fleshy scale leaves with increase in concentration and it was lower to control value. Soluble protein content in cotyledons was higher than in embryo. Soluble protein content on second day

Fig. 69 A : Effect of laser exposure on soluble protein content in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second day and fourth day of sprouting in *A. cepa*

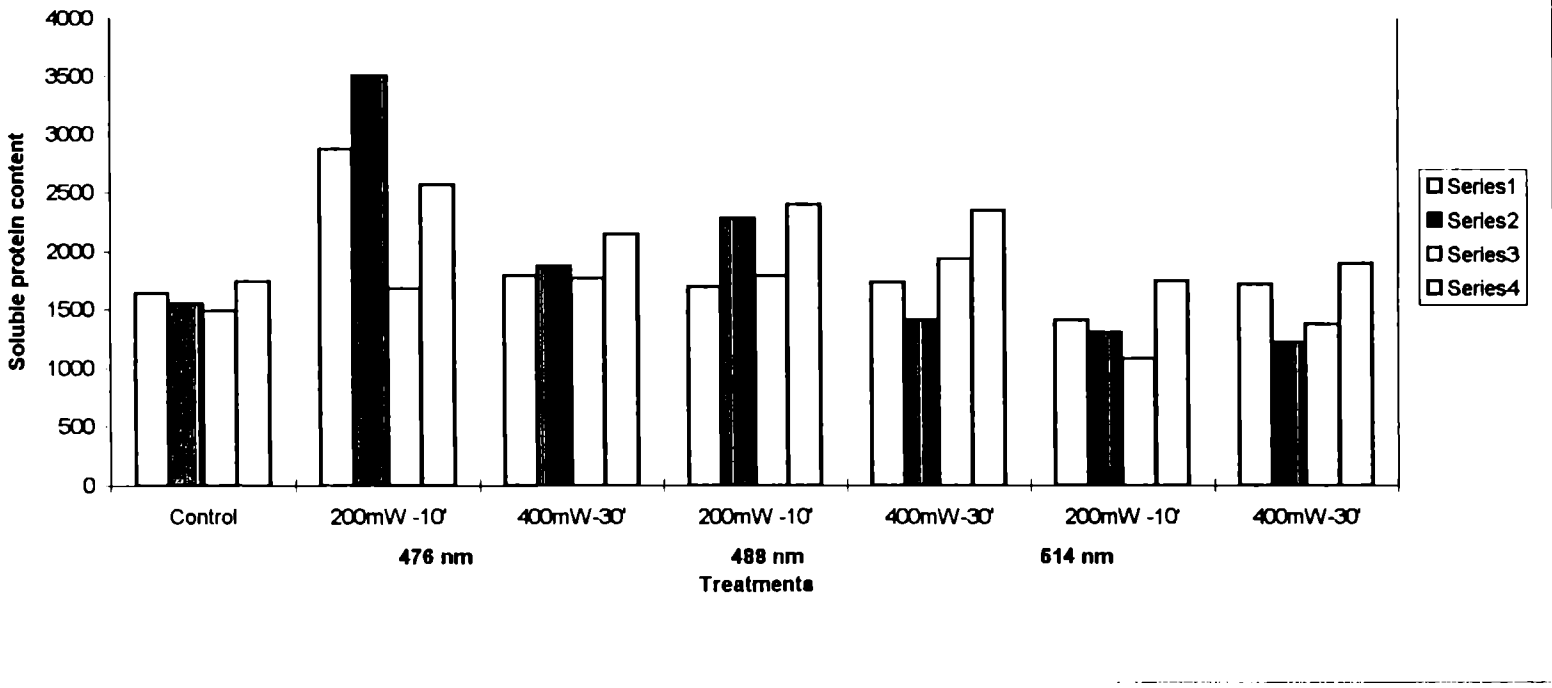


Fig. 70 A : Effect of gamma irradiation on soluble protein content in inner true leaves (ser. 1) and outer fleshy scale leaves (ser. 2) on second day of sprouting in *A. cepa*

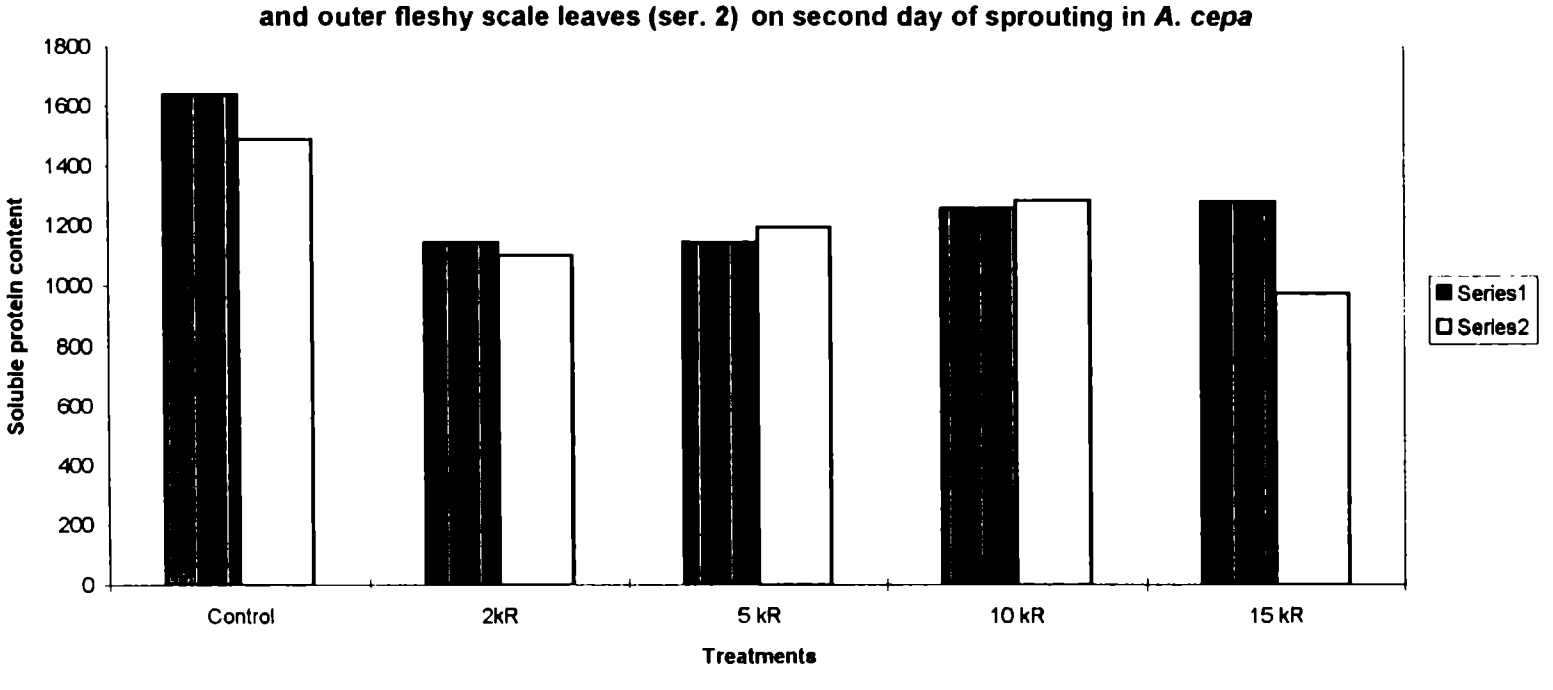


Fig. 71 A : Effect of EMS treatment on soluble protein content in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

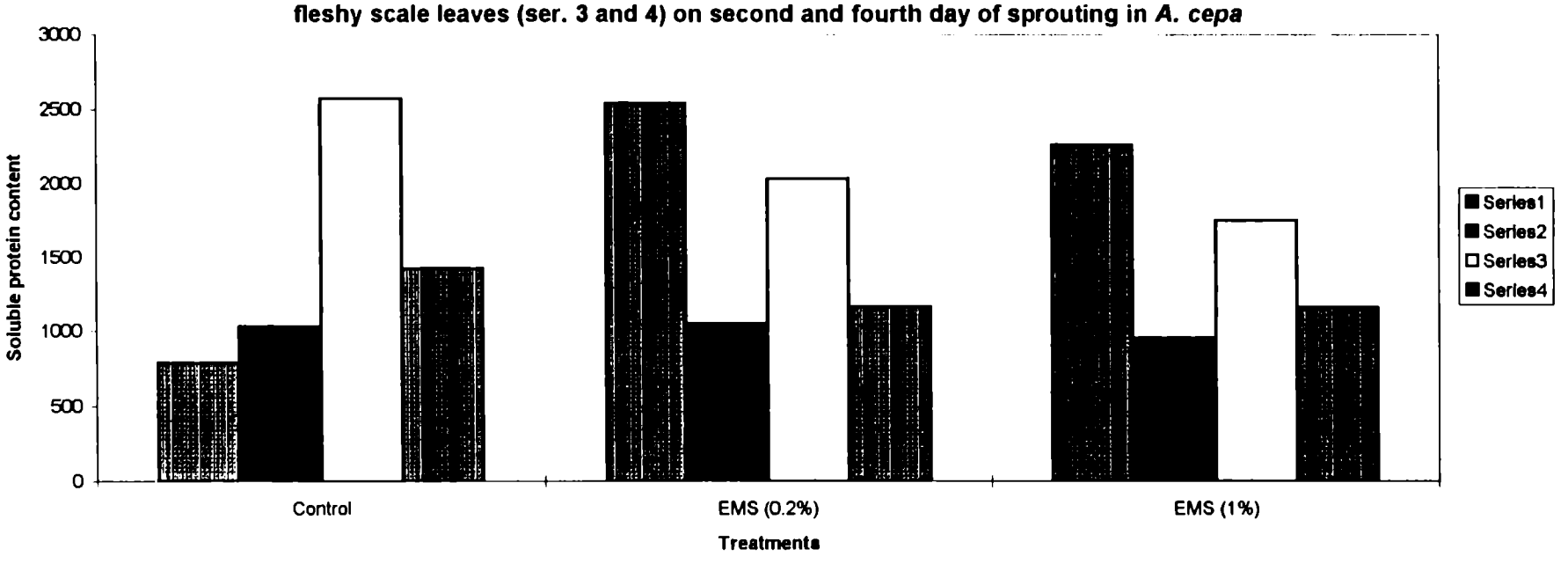
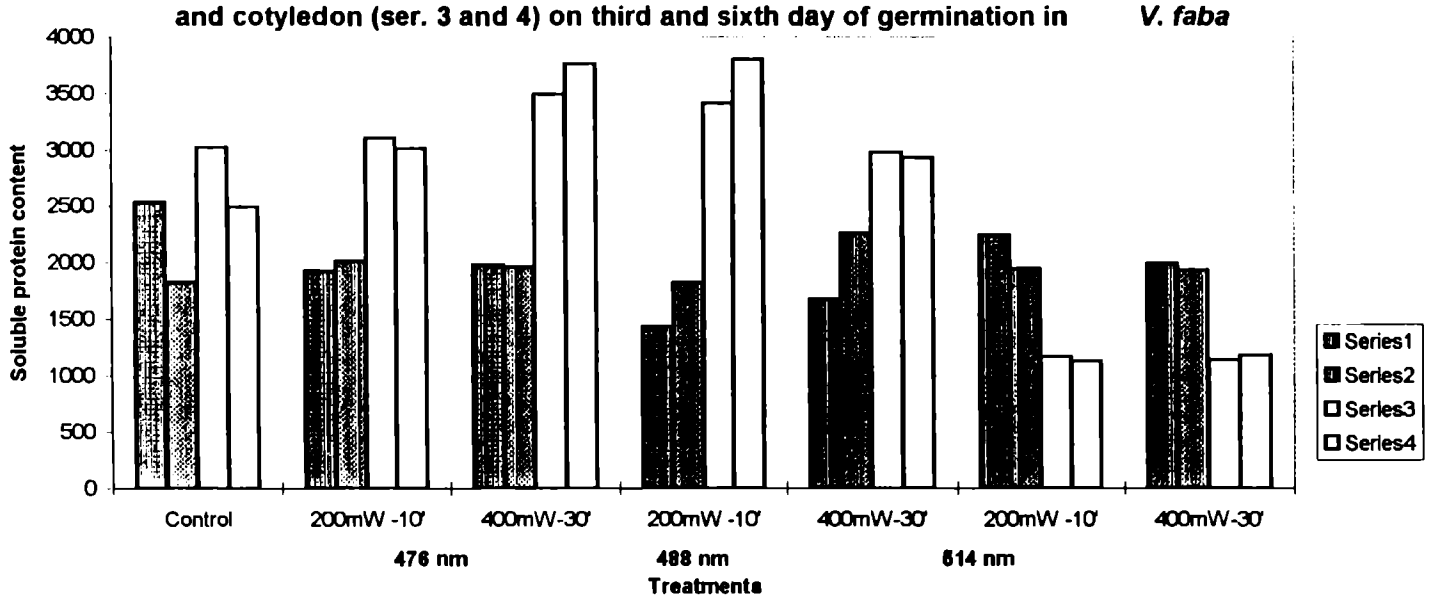


Fig. 88 B : Effect of laser exposure on soluble protein content in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in *V. faba*



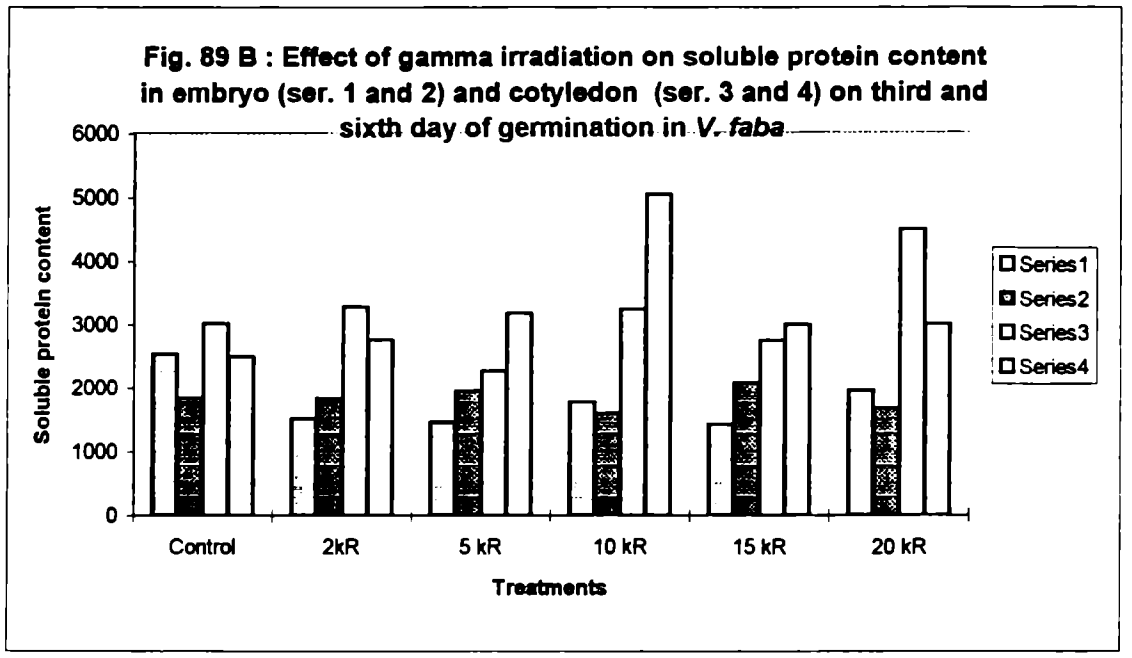


Fig. 89 B : Effect of gamma irradiation on soluble protein content in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in *V. faba*

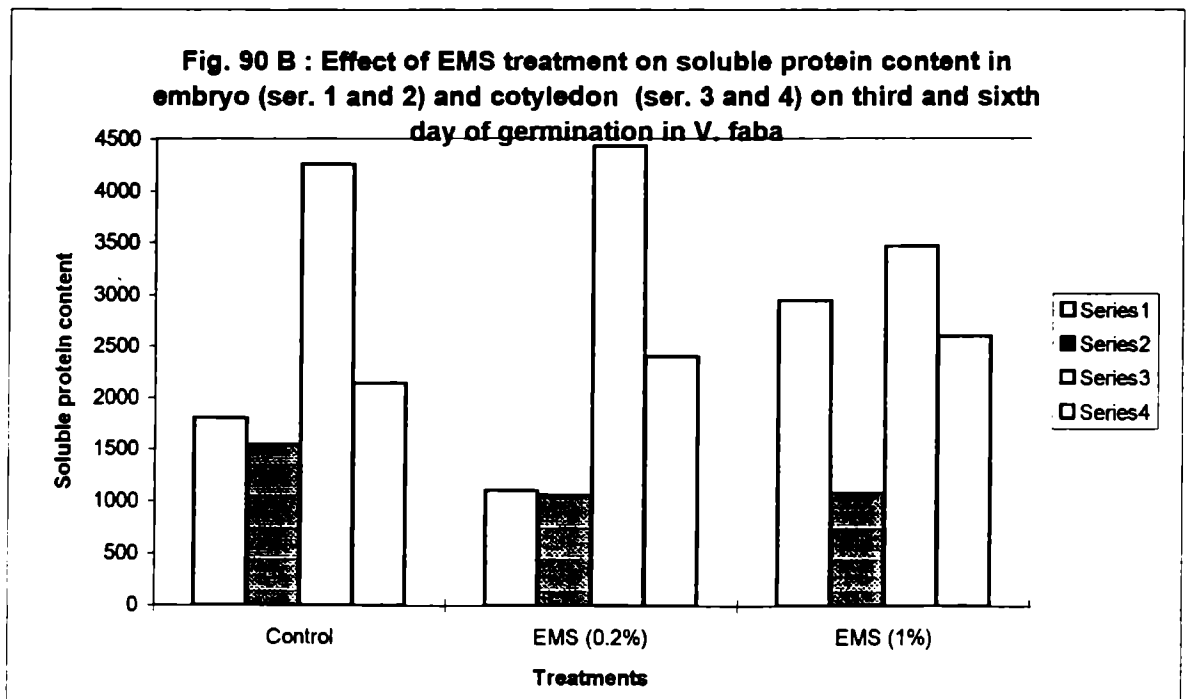


Fig. 90 B : Effect of EMS treatment on soluble protein content in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in V. faba

was higher than on fourth day (Fig.71A App.VIIIA). EMS treatments in *V. faba* also caused decrease in soluble protein content in cotyledon on third day and increase in soluble protein content in cotyledon on sixth day when compared to control. Soluble protein content decreased in embryo in EMS treated samples on sixth day and on third day soluble protein content in embryo decreased in 0.2% and significantly increased in 1% EMS treatment when compared to control. Soluble protein content in cotyledon was higher than in embryo (Fig.90B App.XB).

Comparing the soluble protein content in *A. cepa* and *V. faba*, it was found that laser exposures induced increase in soluble protein content in *A. cepa* and in cotyledon of *V. faba* but 514 nm of laser induced decrease in soluble protein content in *V. faba* cotyledon while the soluble protein content of embryo was decreased on third day and increased on sixth day in *V. faba*. Soluble protein content decreased in *A. cepa* and in embryo of *V. faba* in γ-irradiations but in cotyledon of *V. faba* soluble protein content increased on sixth day and there was no change on third day. EMS treatment caused decrease in soluble protein content in *A. cepa* and variable response in *V. faba*.

4.6.2 Protease activity

In *A. cepa,* laser exposures at 476 and 488 nm caused no significant change in enzyme activity compared to control (Fig.72A App.IXA), but the specific activity showed increase with increase in dose in samples exposed to these two wavelengths. Outer fleshy scale leaves showed higher activity and specific activity than inner true leaves. 514 nm of lasers caused decrease in activity and specific activity with increase in power density (Fig.72A App.IXA). In *V. faba,* laser exposures at 476 nm and 488 nm induced increase

n enzyme activity (Fig. 91B App.XIB) and specific activity in cotyledon and embryo and was higher than control value. An increase in enzyme activity with ncrease in power density was found at 488 nm but at 476 nm the enzyme ctivity decreased with increase in power density. At 514 nm laser exposure caused decrease in activity in embryo and cotyledon ant dt was lower than control (Fig.91B App.XIB). 514 nm also caused decrease in enzyme activity with ncrease in power density. In embryo specific activity showed variation and was igher than control at 476 and 488 nm of laser exposures. Variation in specific ctivity was not found in cotyledon.

γ-irradiations induced decrease in activity (Fig.73A App.IXA) and pecific activity of protease in *A. cepa*. In *V. faba,* γ-irradiations induced no ignificant change in enzyme activity in treated samples but was higher than ontrol. Specific activity in γ-irradiation did not show much change in cotyledon nd was highest in 2kR γ-irradiated samples (Fig.92B App.XIB)

In *A. cepa,* EMS treatment caused no significant variation in nzyme activity in treated samples compared to that of control (Fig.74A pp.IXA). Specific activity in inner true leaves of samples treated with EMS howed decrease with increase in dose on second day and was several fold wer to control. But the specific activity in outer fleshy scale leaves on second ay showed increase with increase in dose and was higher to control value. nner true leaves and outer fleshy scale leaves did not show significant ariation in activity on fourth day. In *V. faba,* protease activity in EMS treated amples did not show much variation in embryo and cotyledon (Fig.93B pp.XIB). However in embryo it was lower than control and in cotyledon similar o control. Specific activity of the enzyme in samples treated with EMS at oncentration 2% was higher and at 1% was lower in embryo compared to

Fig. 72 A : Effect of laser exposure on protease activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

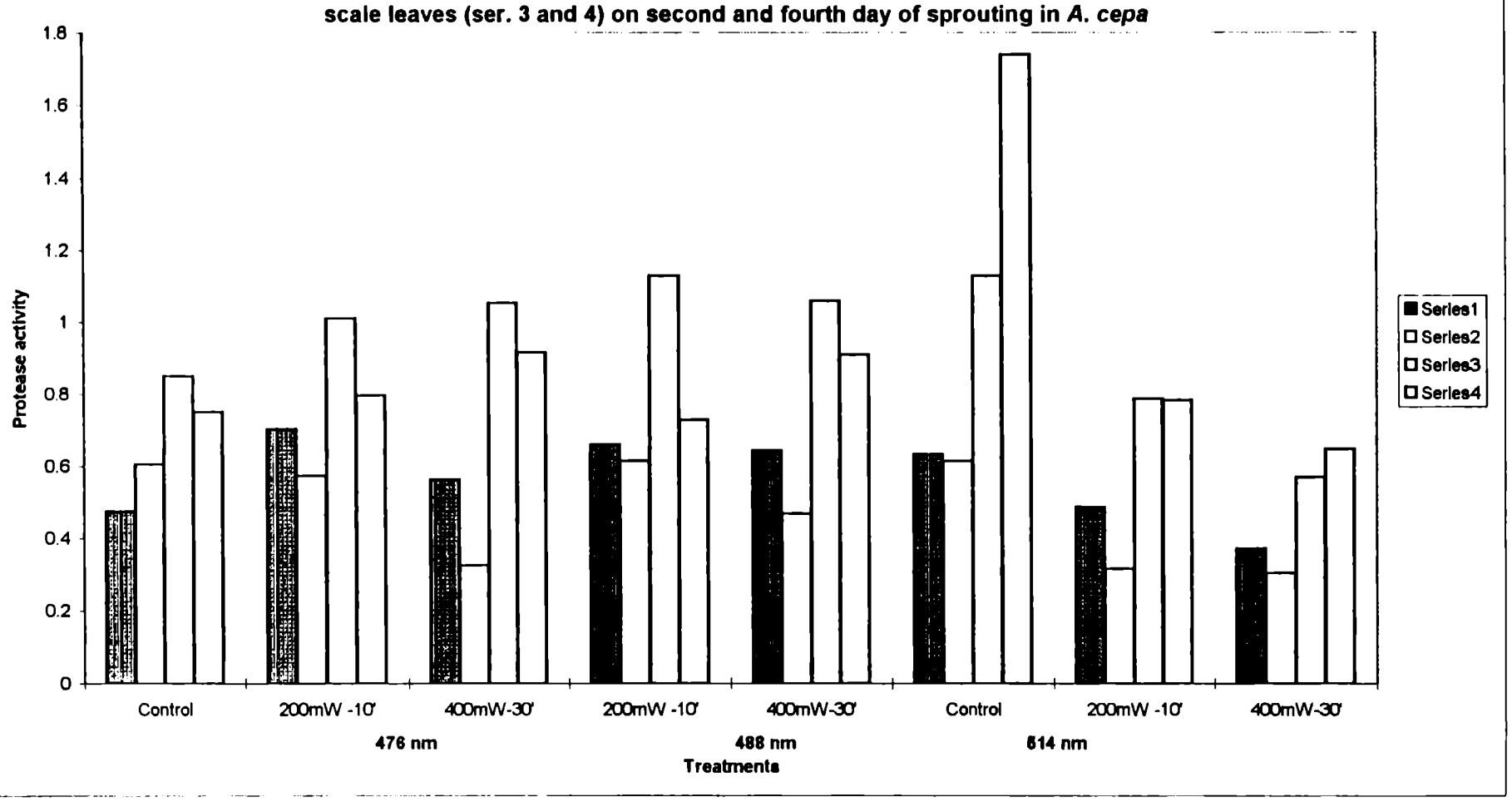


Fig. 73 A : Effect of gamma irradiation on protease activity in inner true leaves (ser. 1) and outer fleshy scale leaves (ser. 2) on second day of sprouting in *A. cepa*

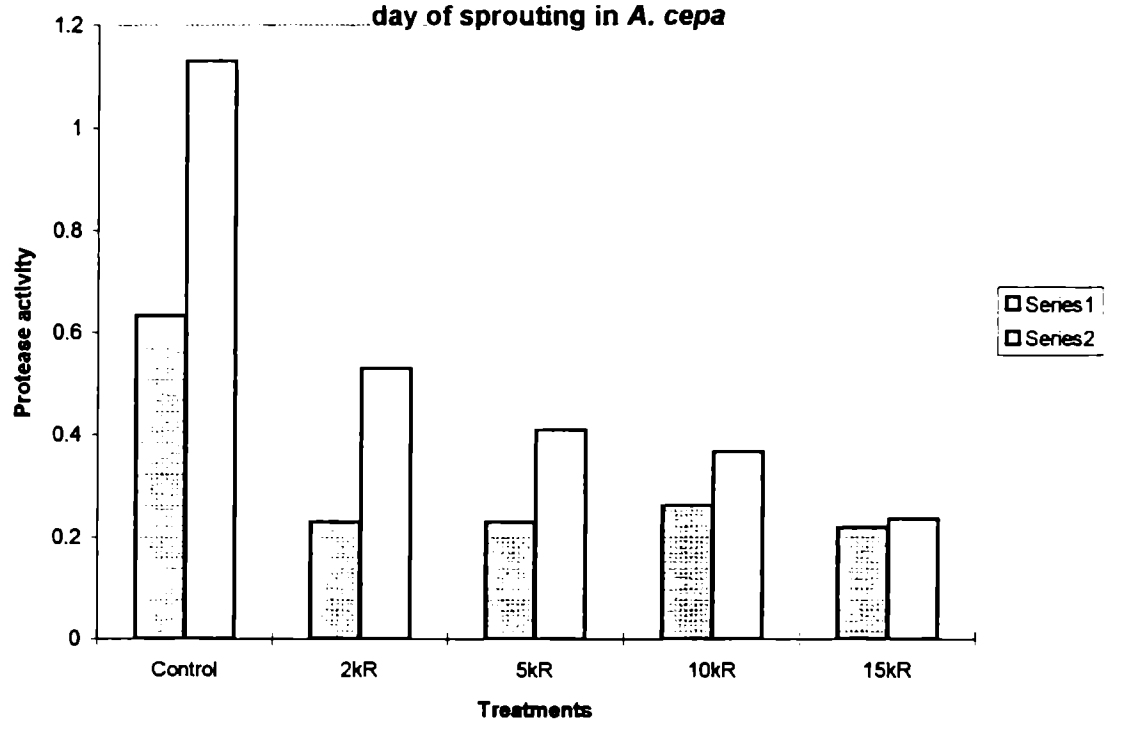


Fig. 74 A : Effect of EMS on protease activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

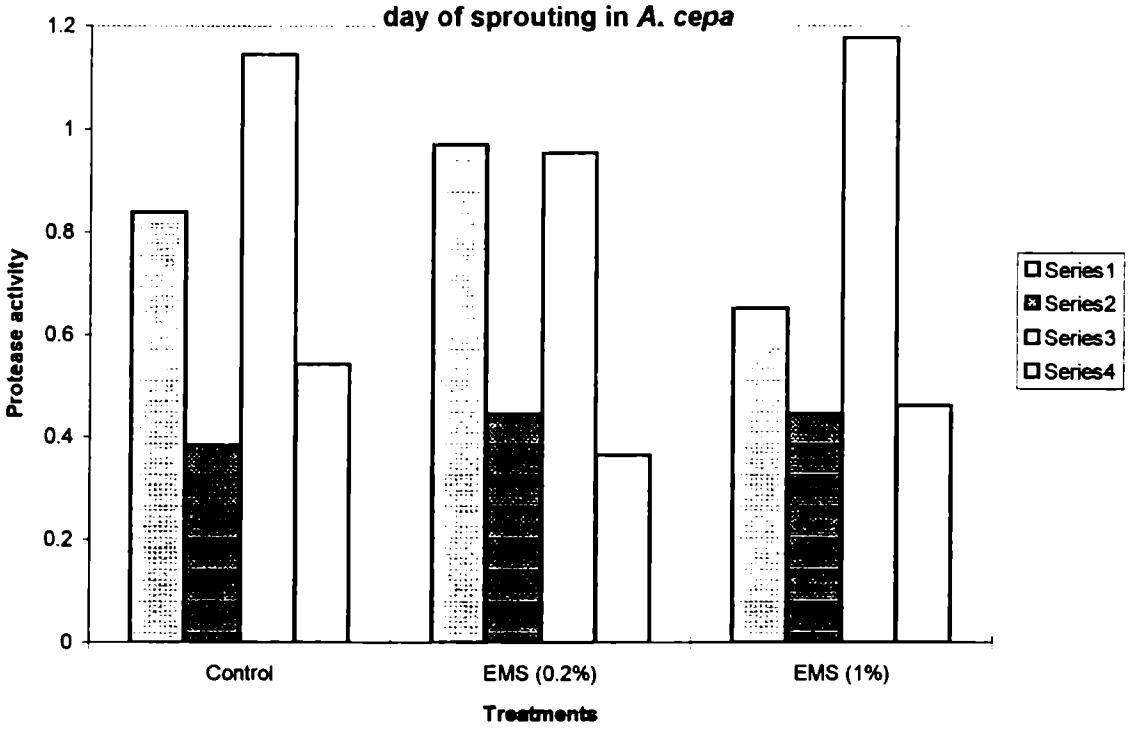


Fig. 91 B : Effect of laser exposure on protease activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in V. faba

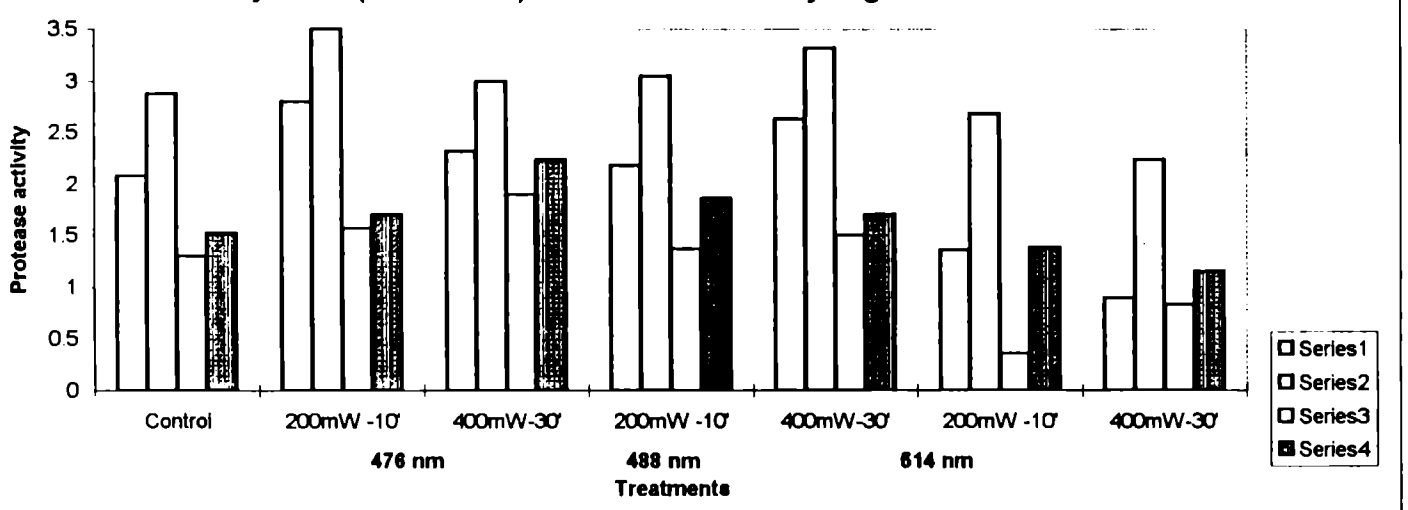


Fig. 92 B : Effect of gamma irradiation on protease activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in V. faba

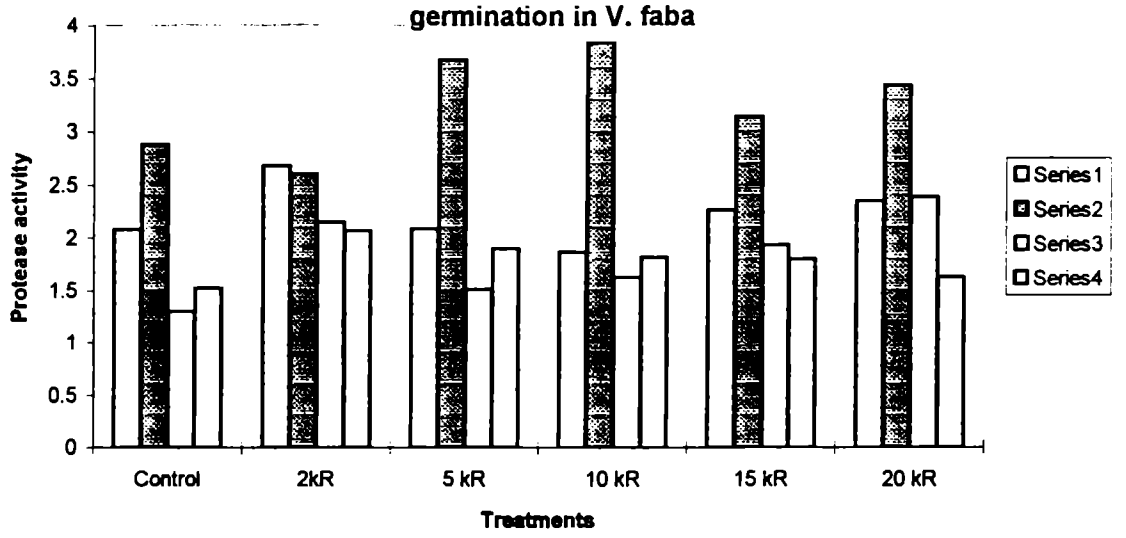
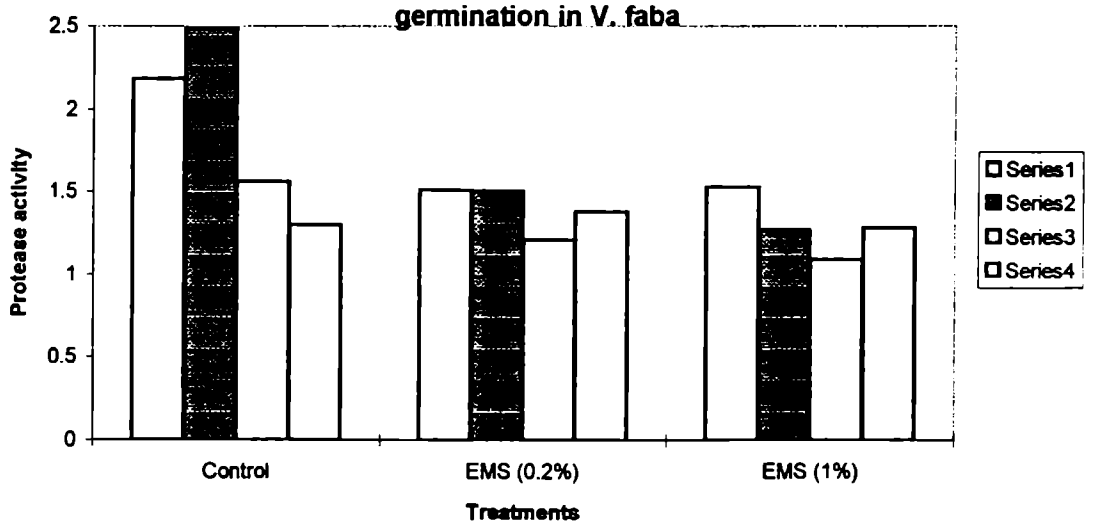


Fig. 93 B : Effect of EMS treatment on protease activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on third and sixth day of germination in V. faba



control. On second and fourth days it was lower at 0.2% and higher at 1% than that of control. In cotyledon no significant difference in specific activity between control and treatments was observed.

In the two plant species, lasers of 514 nm caused a decrease in enzyme activity with increase in power density. Lasers of 476 and 488 nm induced an increase in activity in *V. faba* embryo samples over control although in *V. faba* cotyledon samples and in *A. cepa* no significant change in activity was observed compared to control samples. Although γ - irradiations caused a dose dependent decrease in activity in *A. cepa*, no significant change in activity was observed in *V. faba*. Treatment with EMS did not induce significant change in enzyme activity in either plant species, although specific activity showed tissue specific and day specific changes in both plant species.

4.6.3 Amylase activity

In *A. cepa,* laser exposures at 476, 488 and 514 nm did not show change in amylase activity compared to control (Fig.75A App.XA). In laser exposed samples specific activity was higher than control and an increase of this with dose was noticed at 488 and 514 nm samples. In *V. faba* significant difference in amylase activity in laser exposed samples compared to control samples was not noticed. 476 and 514 nm of laser exposures induced a decrease in activity with increase in dose (Fig.94B App.XIIB). On second day specific activity in the embryo in laser treated samples was lower than the control but on fourth day it was similar to the control. Specific activity in cotyledon of laser treated samples showed no significant difference compared to the control.

In *A. cepa* γ-irradiation caused dose dependent increase in activity and specific activity compared to that of control (Fig.76A App.XA). Specific activity in lower doses of γ-irradiations was higher in inner true leaves but the specific activity was higher in outer fleshy scale leaves in higher doses of γ-irradiations. In *V. faba,* γ-irradiations induced slightly higher enzyme activity in cotyledon and embryo than control but dose dependent decrease in activity was seen in embryo (Fig.95B App.XIIB). 2 kR γ-irradiated samples showed maximum activity. Increase in specific activity also was noticed in γ - irradiated samples. 2 kR γ-irradiated samples showed maximum specific activity.

EMS treatment in *A. cepa* caused significant decrease in enzyme activity at 0.2% although not much difference was noticed at 1% when compared to control in the inner true leaves. Outer scale leaves at 0.2% treatment showed significant increase in enzyme activity on second day when compared to control (Fig.77A App.XA). Other treatments did not cause much differences in activity. Specific activity in samples given 0.2% EMS treatment showed significant decrease on second day and not much difference on fourth day in inner true leaves when compared to control. The outer scale leaves on second day showed significant decrease in specific activity compared to that of control. EMS treatments did not show much difference in speicific activity in inner true leaves and outer fleshy scale leaves on the fourth day of treatment. In *V. faba* EMS caused decrease in amylase activity in cotyledon and embryo on fourth day of treatment but on second day EMS treatment induced increase in enzyme activity at 0.2% and decrease at 1% in embryo while in cotyledon decrease in activity at 0.2% and increase at 1% when compared to control was found (Fig.96B App.XIIB). Specific activity showed no significant change in

Fig. 75 A : Effect of laser exposure on amylase activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

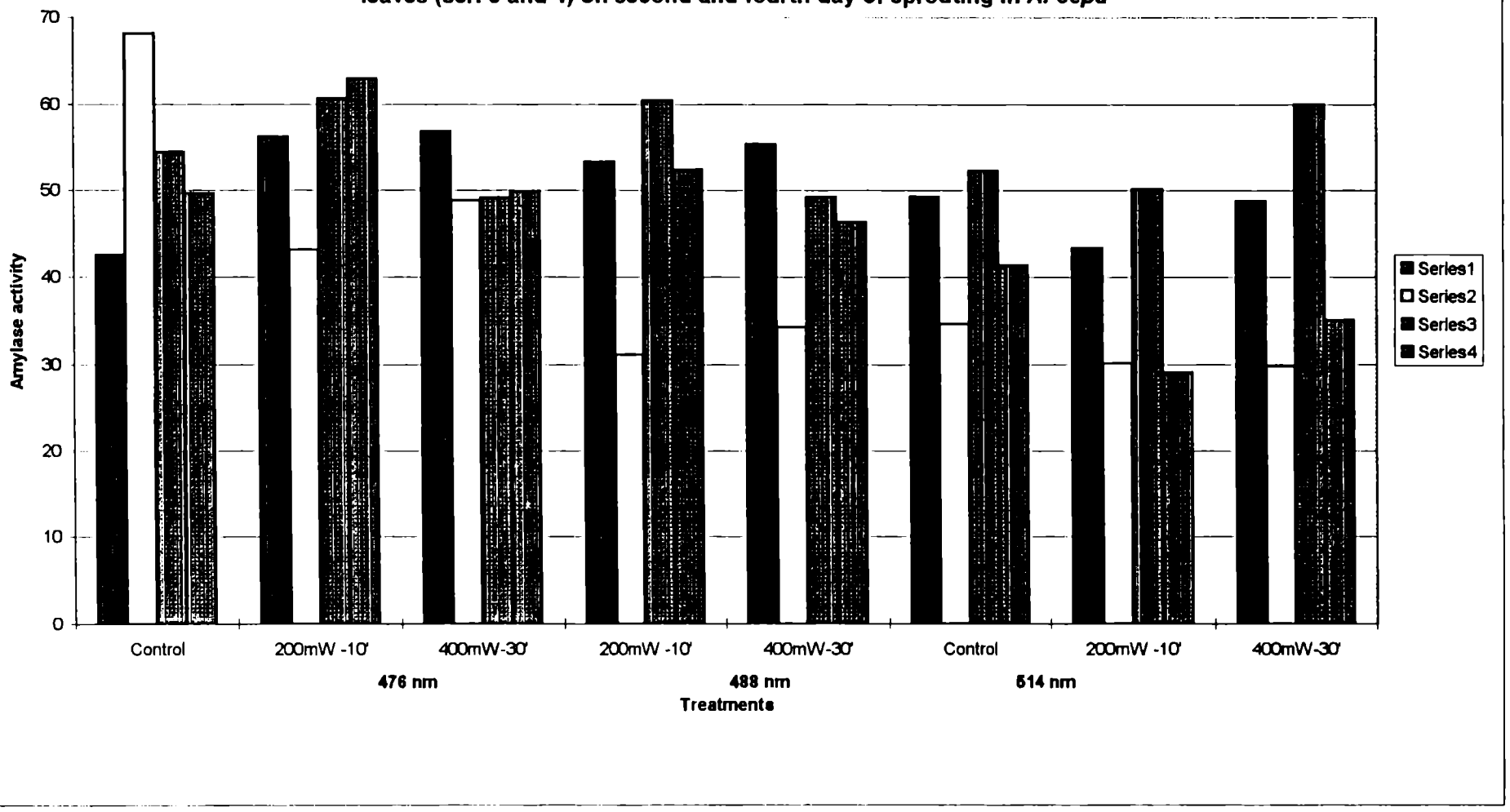


Fig. 76 A : Effect of gamma irradiation on amylase activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

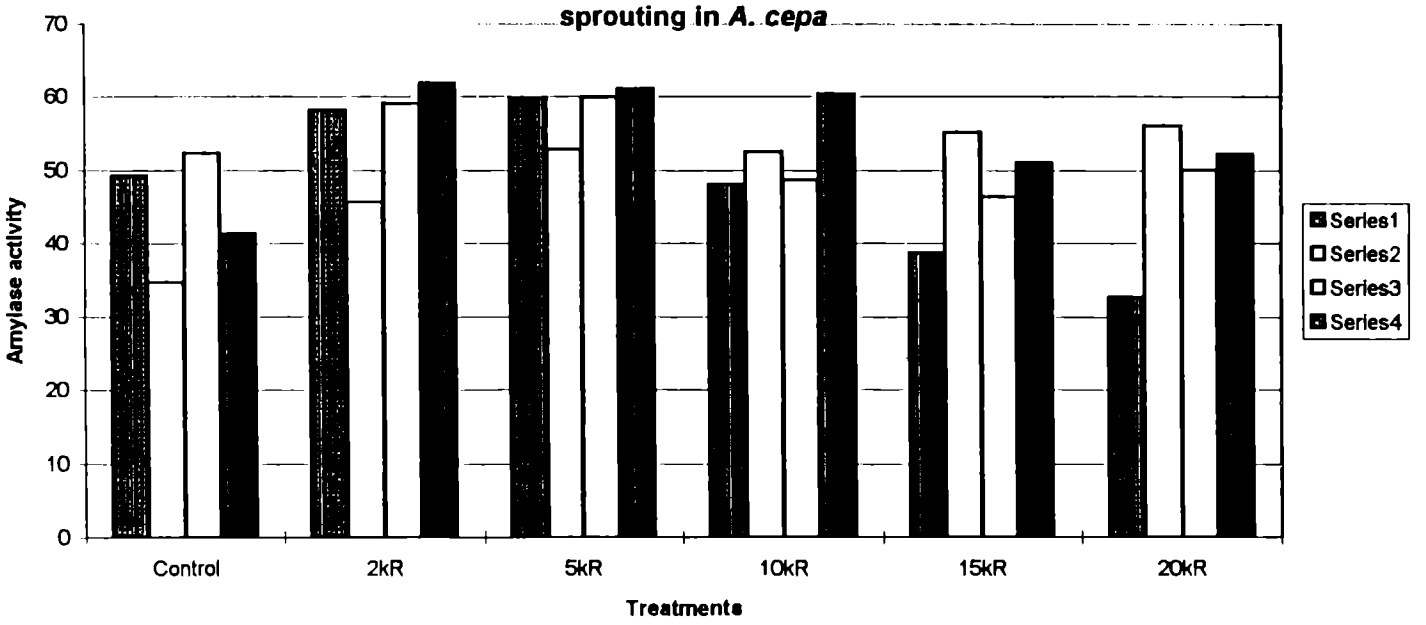


Fig. 77 A : Effect of EMS treatment on amylase activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day in *A. cepa*

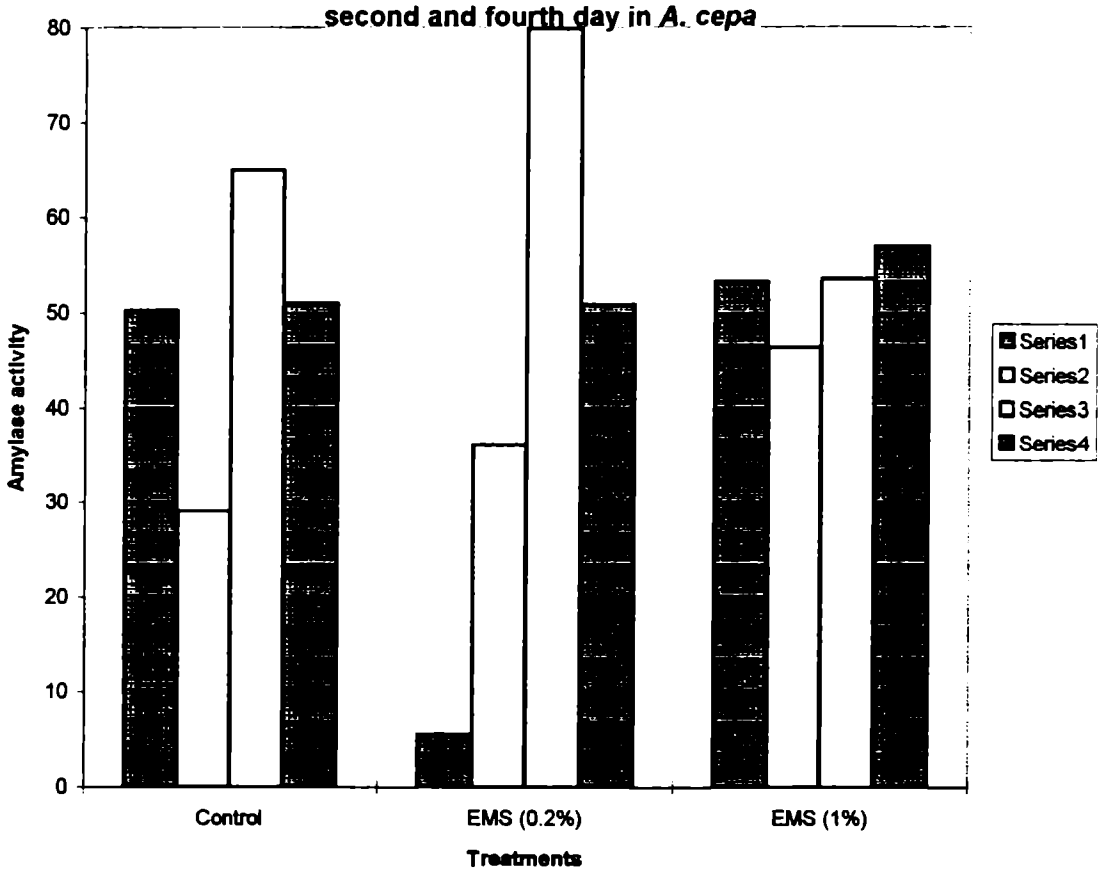


Fig. 94 B : Effect of laser exposure on amylase activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on second and fourth day of germination in *V. faba*

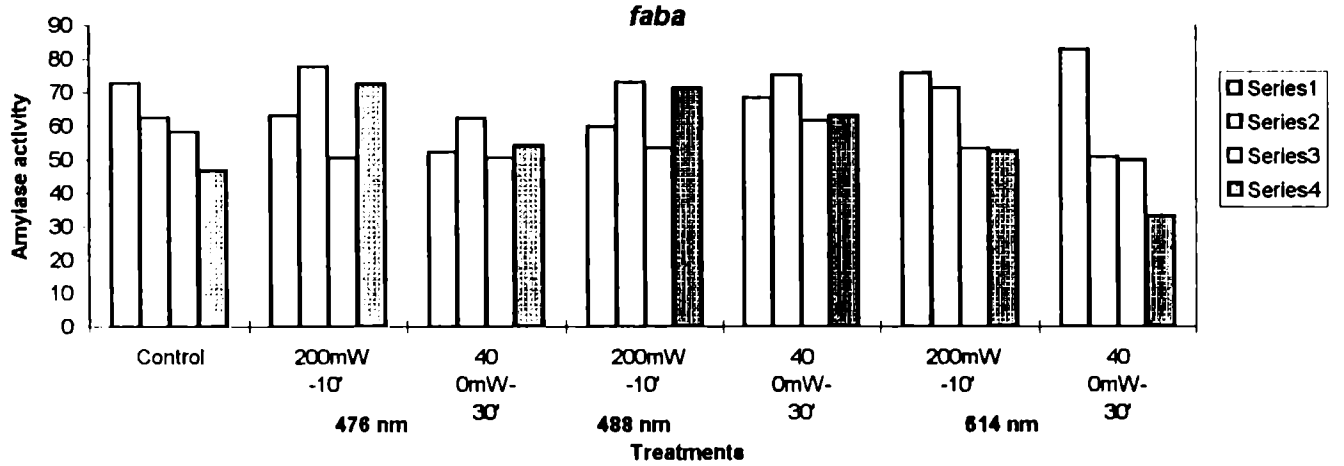


Fig. 95 B : Effect of gamma irradiation on amylase activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on second and fourth day of germination in *V. faba*

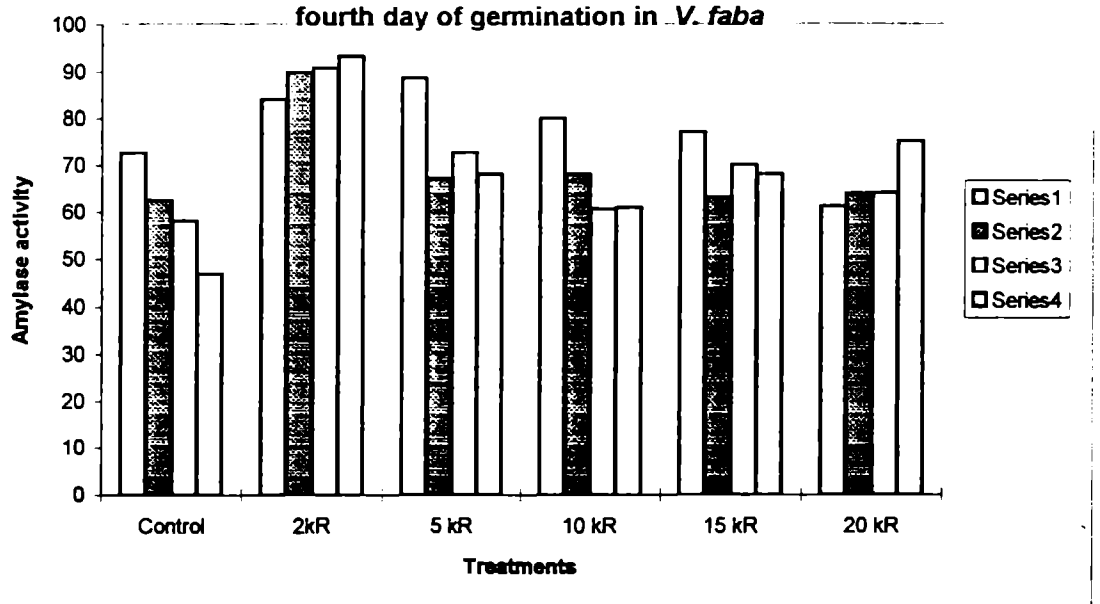
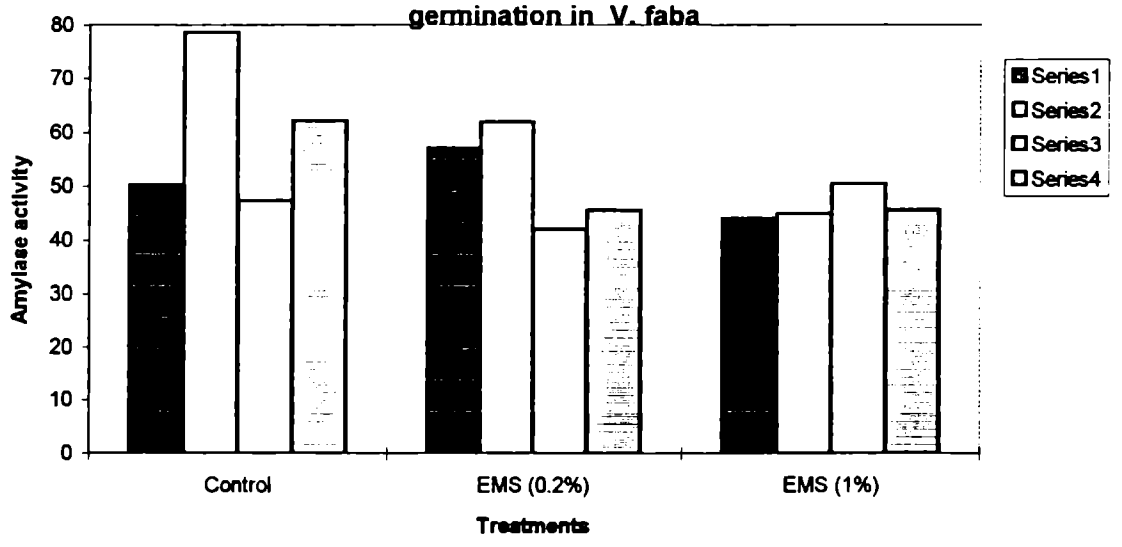


Fig. 96 B : Effect of EMS treatment on amylase activity in embryo (ser. 1 and 2) and cotyledon (ser. 3 and 4) on second and fourth day of germination in *V. faba*



second and fourth day.

Comparing the response of the two plant species, it was seen that lasers of 476, 488 and 514 nm did not cause a significant change in amylase activity in either plant species. The specific activity of amylase was however higher than that of control in *A. cepa* samples exposed to 488 nm and 514 nm but the specific activity was lower than that of control in *V. faba* laser treated samples. In both plant species, enzyme activity and specific activity was higher in γ - treated samples than the control samples. EMS induced tissue specific and day specific changes in enzyme activity and specific activity in both plant species.

4.6.4 Peroxidase activity

Peroxidase activity is significantly absent or very low in outer fleshy scale leaves as compared to the inner true leaves in onion bulbs. Laser exposures caused no significant change in enzyme activity in inner true leaves in *A. cepa* bulbs compared to control samples (Fig.78A App.XIA). In *V. faba,* laser exposure at 514 nm induced increase in peroxidase activity in cotyledon. Peroxidase activity was lower than control in the cotyledon and embryo in all other laser exposures (Fig.97B App.XIIIB).

γ - irradiations in *A. cepa* caused transient increase in peroxidase acitivity in irradiated samples compared to control and no dose dependence was found (Fig.79A App.XIA). In *V. faba,* γ - irradiations induced peroxidase activity higher than control in the embryo at 5kR irradiation but the activity was similar to control in the embryo of 15 kR and 20 kR irradiated samples. The activity

Fig. 78 A : Effect of laser exposure on peroxidase activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

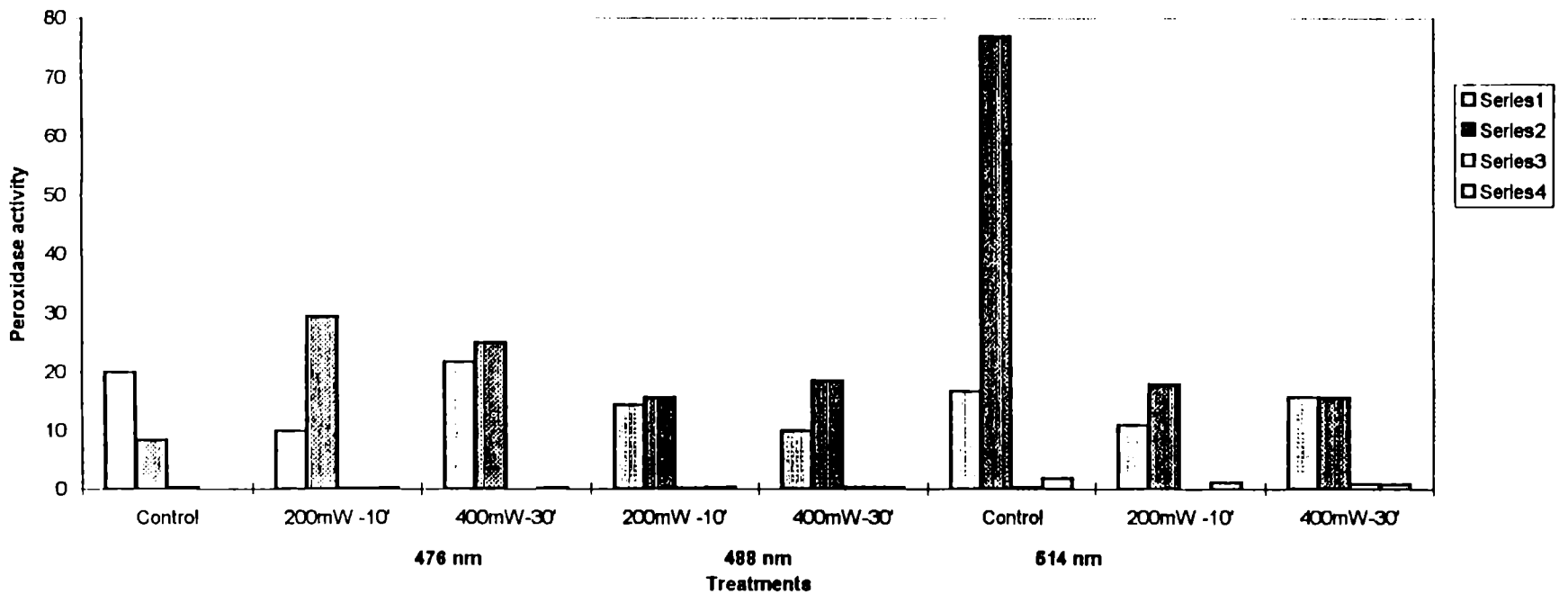


Fig. 79 A : Effect of gamma irradiation on peroxidase activity in inner true leaves (ser. 1 and 2) and outer fleshy scale leaves (ser. 3 and 4) on second and fourth day of sprouting in *A. cepa*

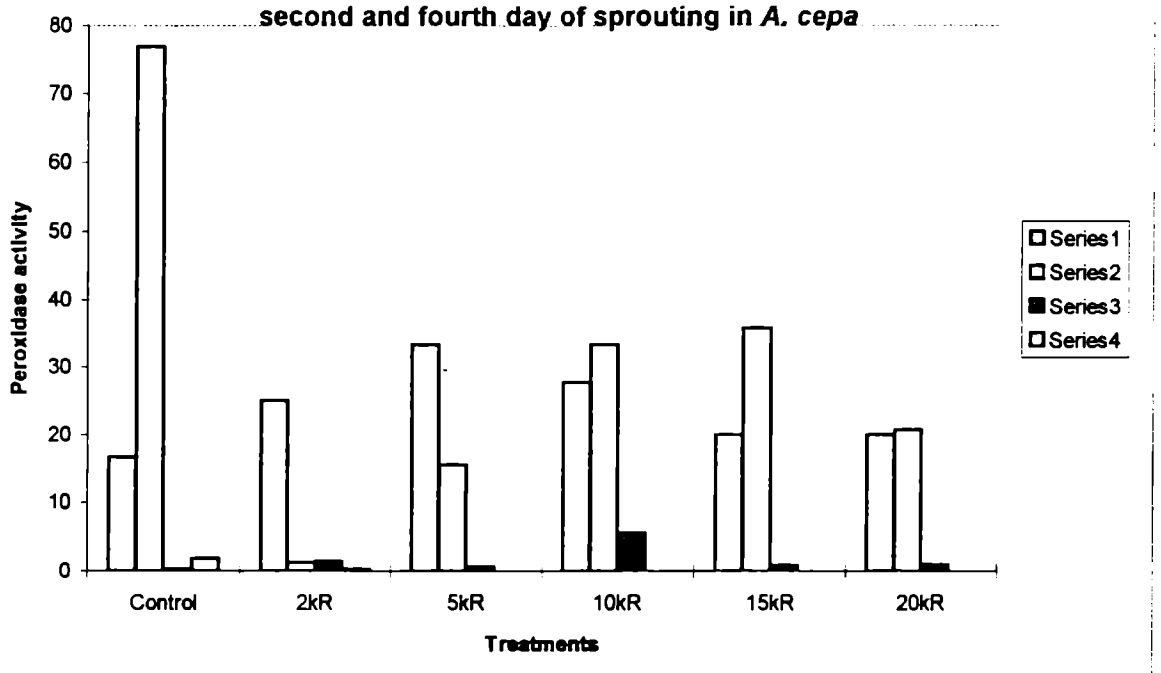


Fig. 80 A : Effect of EMS treatment on peroxidase activity in inner true leaves (ser. 1) and outer fleshy scale leaves (ser. 2) on fourth day of sprouting in A. cepa

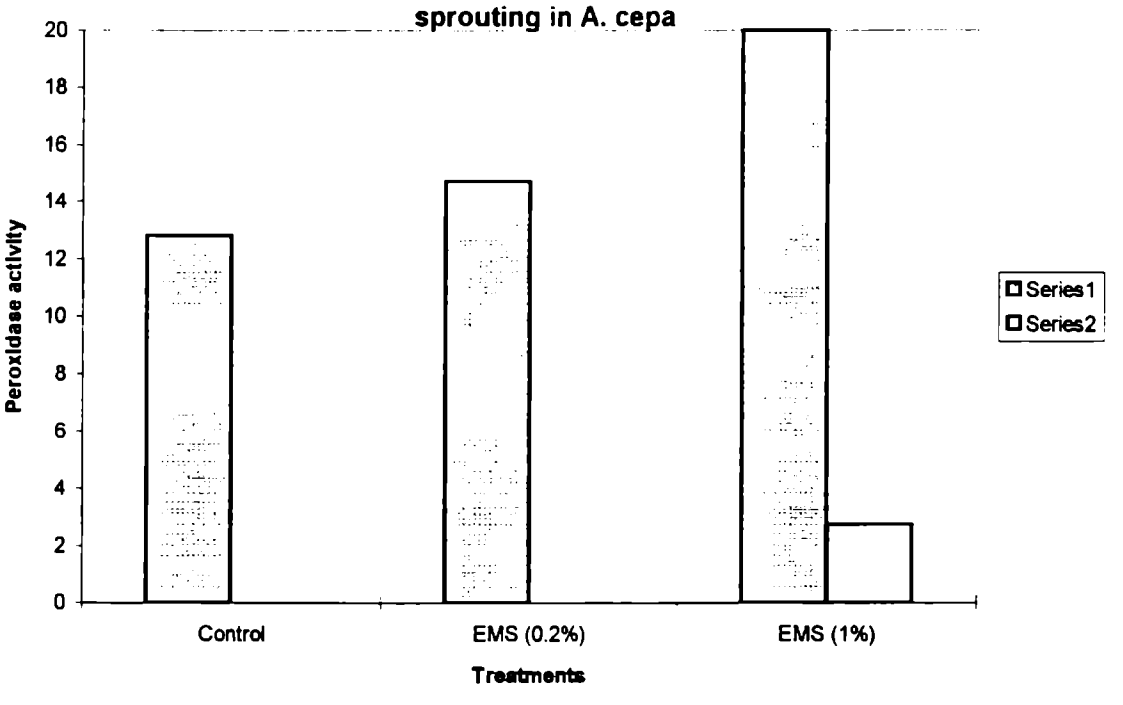


Fig. 81 A : Effect of laser exposure on catalase activity in inner true leaves (ser. 1 and 2) on second and fourth day of sprouting in A. cepa

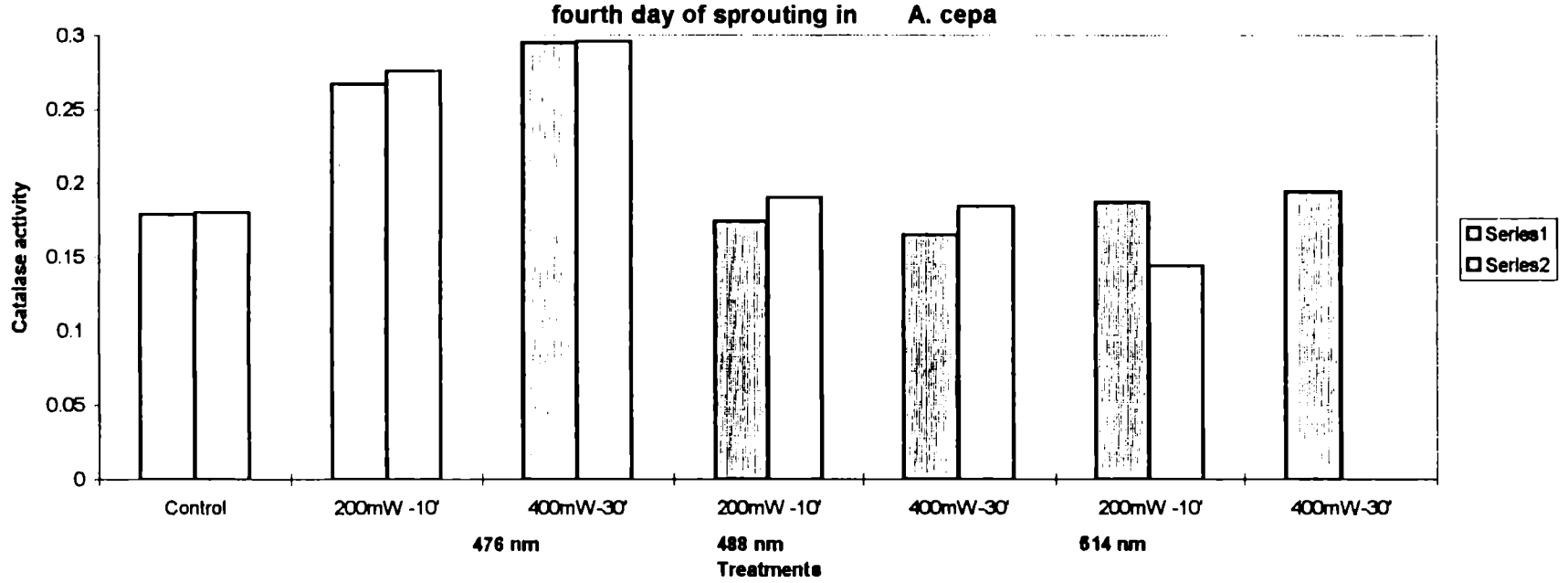


Fig. 97 B : Effect of laser exposure on peroxidase activity in embryo (ser. 1) and cotyledon (ser. 2) on fifth day of germination in *V. faba*

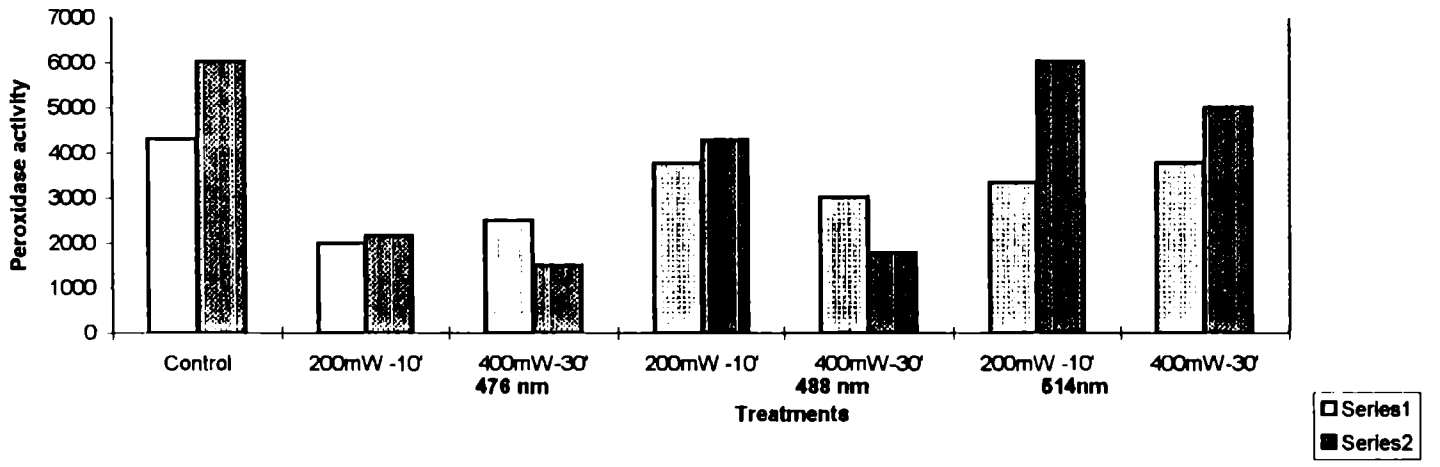


Fig. 98 B : Effect of gamma irradiation on peroxidase activity in embryo (ser. 1) and cotyledon (ser. 2) on fifth day of germination in *V. faba*

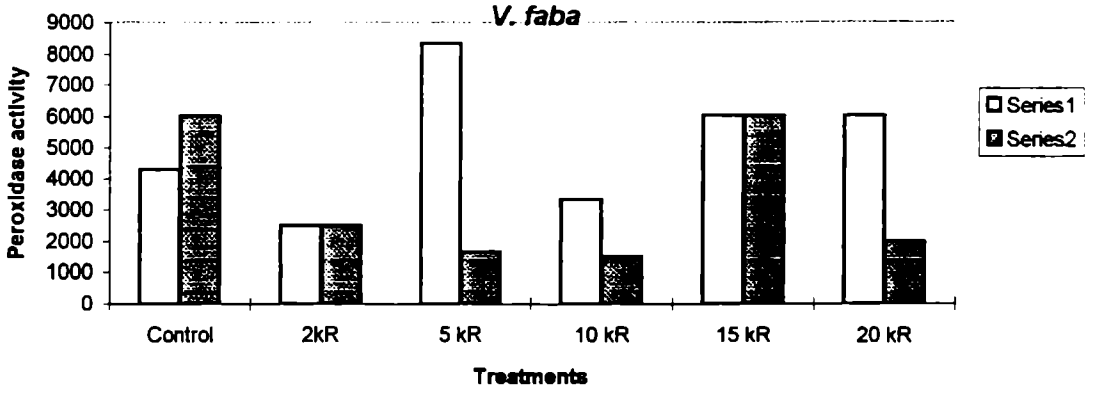
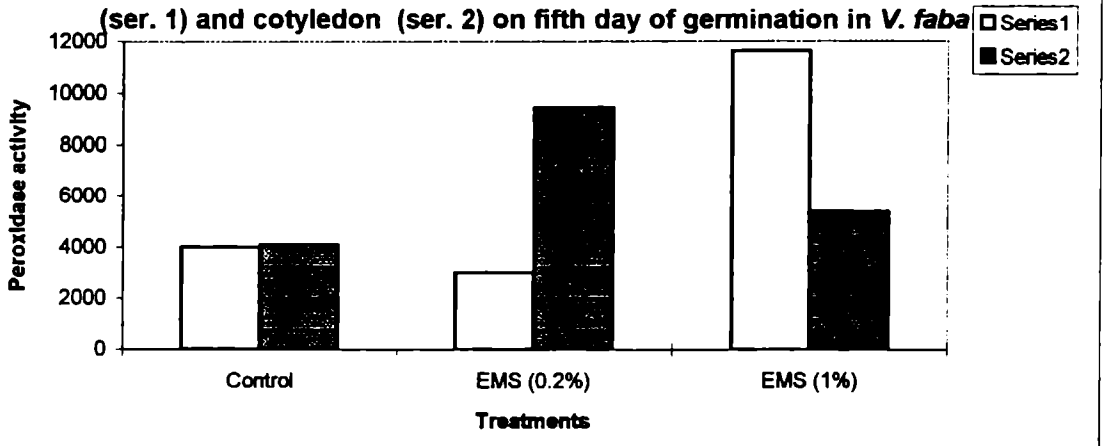


Fig. 99 B : Effect of EMS treatment on peroxidase activity in embryo (ser. 1) and cotyledon (ser. 2) on fifth day of germination in *V. faba*



was lower than control in 2 and 10 kR γ - irradiated samples in embryo and in cotyledon of all γ- irradiations (Fig.XIIIB App.98B).

In *A. cepa*, EMS treatment caused an increase in peroxidase activity in inner true leaves with increase in treatment concentration in treated bulbs compared to control (Fig.80A App.XIA). In *V. faba* also a transient increase in peroxidase activity was found in embryo with increase in treatment concentration in EMS treated samples compared to control (Fig.99B App.XIIIB). But in cotyledon enzyme activity was lower than control in 0.2% and higher than control in 1% EMS treated samples.

The two plant species differed significantly in that peroxidase activity was seen only in selected tissues (viz. inner true leaves) in *A. cepa* although it was present in all tissues in *V. faba*. Laser exposures did not cause any significant change in enzyme activity in *A. cepa* but in *V. faba* lasers of 514 nm caused increase in peroxidase activity in cotyledon although all other laser exposures caused a decrease in activity in both embryo and cotyledons. γ - irradiation caused a transient increase in activity in *A. cepa* but a variable response in *V. faba*. EMS treatments induced enzyme activity higher than control in both plant species.

4.6.5 Catalase activity

In *A. cepa,* catalase activity was significantly absent in the outer fleshy scale leaves compared to the inner true leaves as in the case of peroxidase activity. Laser exposures at 488 nm and 514 nm caused no significant change in enzyme activity but at 476 nm it caused increase in activity with dose, when compared to control (Fig.81A App.XIIA). In *V. faba,*

catalase activity was absent in embryo and found only in cotyledon of both control and treated samples. Laser exposures of 476 nm and 514 nm decreased enzyme activity with increase in power density but at 488 nm the activity increased with increase in power density (Fig.100B App.XIIB).

In *A. cepa*, γ - irraditions induced increase in enzyme activity in irradiated samples compared to control but no dose dependence was found (Fig.82A App.XIIA). In *V. faba*, γ - irradiations induced decrease in enzyme activity compared to control and varied in different doses. 10kR and 15kR irradiated samples showed maximum activity (Fig.101B App.XIVB).

In *A. cepa,* EMS treatments caused increase in catalase activity on second day compared to control in inner true leaves and the increase was not dose dependent but on fourth day it was higher than control in 0.2% and lower than control at 1% EMS treatment (Fig.83A App.XIIA). In *V. faba* catalase activity in cotyledon was higher than control in 0.2% and lower than control in 1% EMS treatments but in embryo at 1% of EMS treatment the activity was lesser than control (Fig.102B App.XIVB).

The two plant species showed similarity in that catalase activity was present in inner true leaves in *A. cepa* and in embryo in *V. faba* and was absent in outer fleshy scale leaves in *A. cepa* and in cotyledon in *V. faba*. Laser exposures of 488 and 514 nm caused no significant change but 476 nm induced dose dependent increase in enzyme activity in *A. cepa* while in *V. faba* laser exposures of 476 and 514 nm caused decrease in enzyme activity but 488 nm caused increase in enzyme activity and the change in activity was dose dependent. γ - irraditions induced increase in enzyme activity in *A. cepa* but a decrease in enzyme activity in *V. faba.* EMS treatment induced variable response in enzyme activity in both plant species.

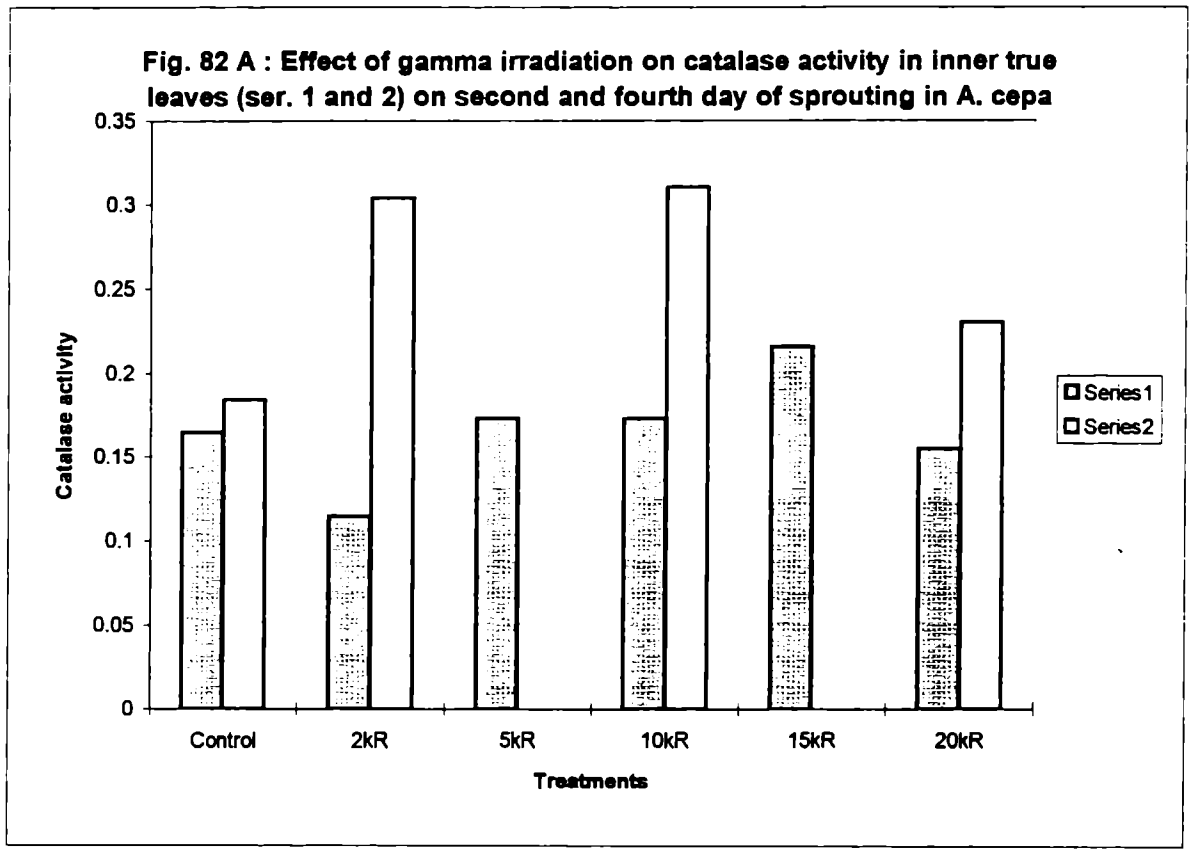


Fig. 82 A : Effect of gamma irradiation on catalase activity in inner true leaves (ser. 1 and 2) on second and fourth day of sprouting in A. cepa
Catalase activity
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Control
2kR
5kR
10kR
15kR
20kR
Treatments
Series1
Series2

Fig. 83 A : Effect of EMS treatment on catalase activity in inner true leaves (ser. 1 and 2) on second and fourth day of sprouting in A. cepa

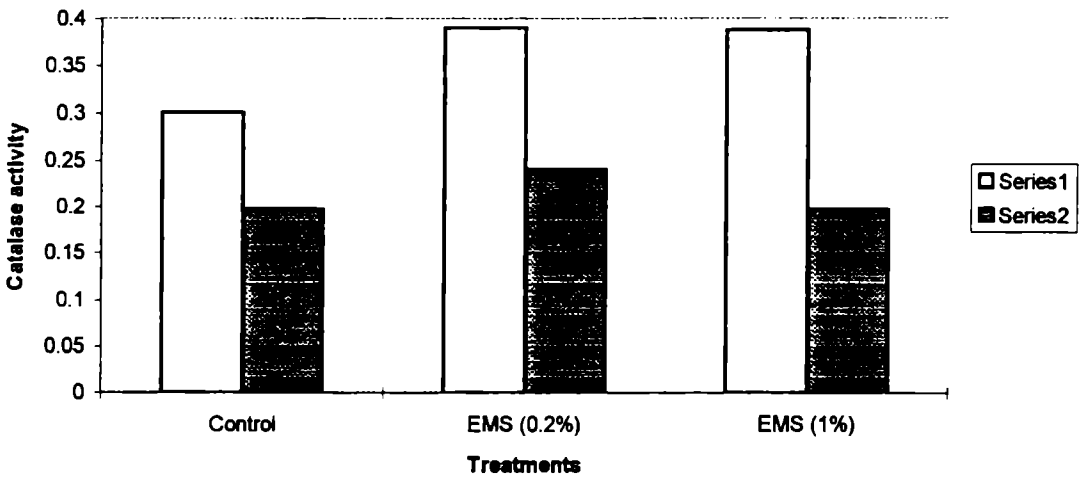


Fig. 100 B : Effect of laser exposure on catalase activity in cotyledon on fifth day of germination in V. faba

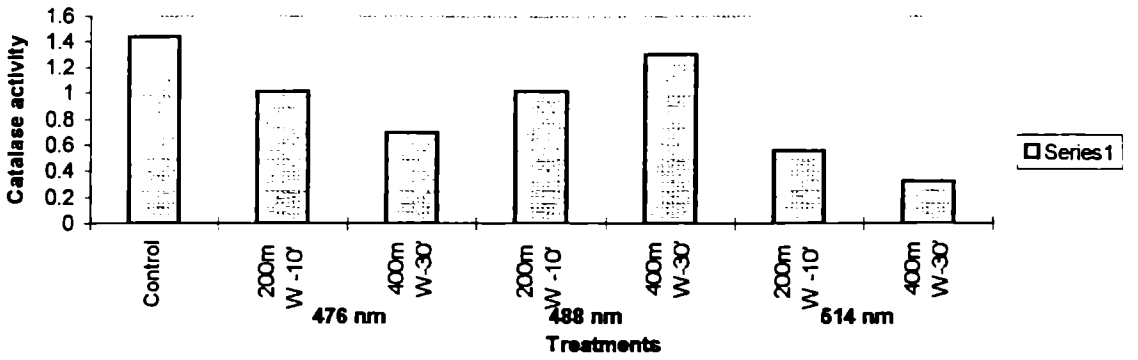


Fig. 101 B : Effect of gamma irradiation on catalase activity in cotyledon on fifth day of germination in V. faba

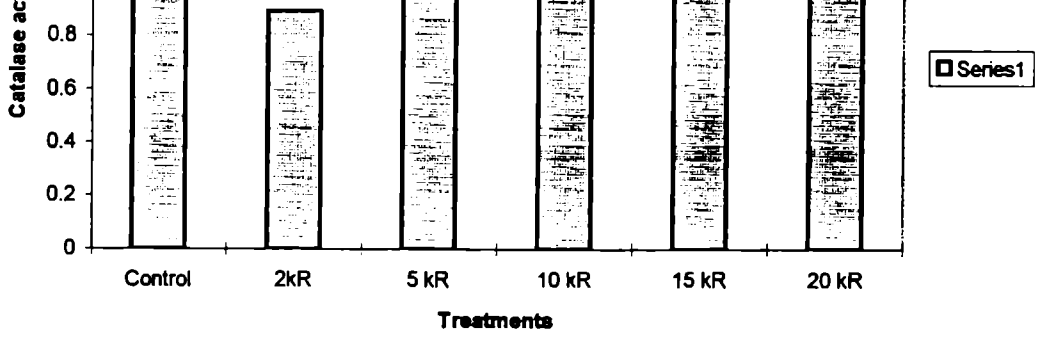
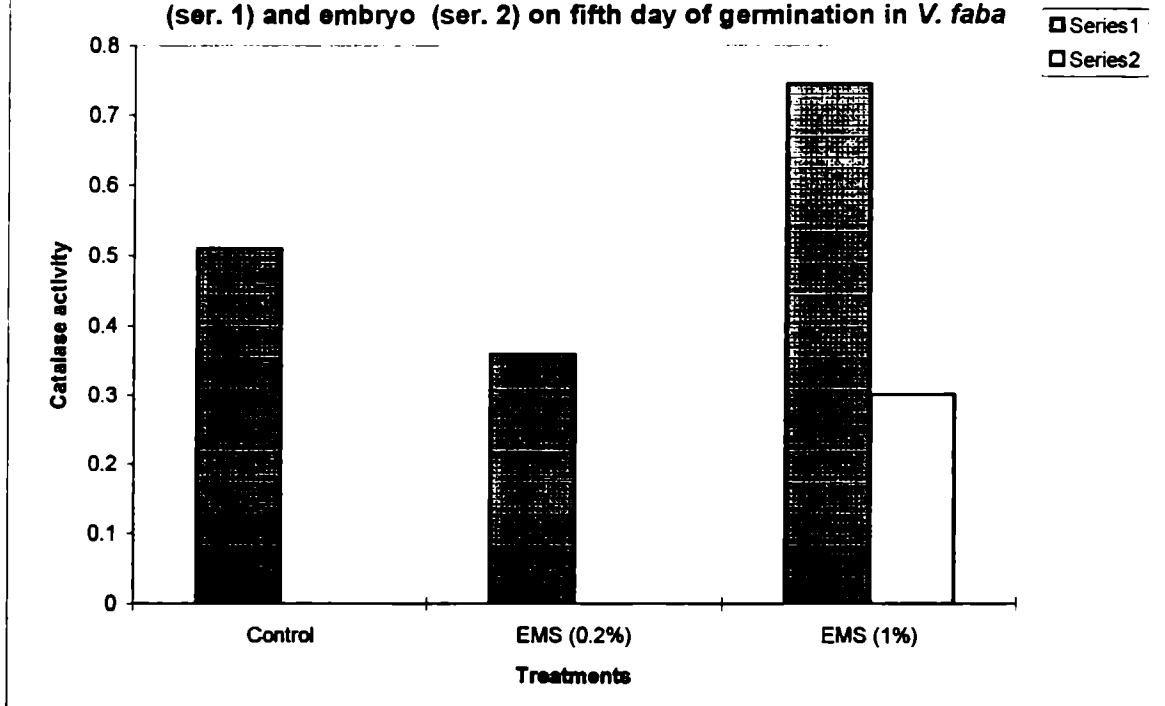


Fig. 102 B : Effect of EMS treatment on catalase activity in cotyledon (ser. 1) and embryo (ser. 2) on fifth day of germination in *V. faba*



DISCUSSION

5.1 Lasers of higher wavelengths (488 and 514 nm) caused aberration in the early stages of mitotic cell division whereas lasers of lower wavelengths (476 nm) caused more aberration in the later stages of mitotic cell division.

The early stages of mitotic cell division are prophase and metaphase. The most important type of chromosomal aberration observed at prophase was clumping of chromosomes and at metaphase was stickiness and nondisjunction of chromosomes.

In laser exposures of *A. cepa,* the frequency of chromosome clumping was similar at 476 and 488 nm and was several fold lower than at 514 nm. In *V. faba,* clumping frequencies were lowest in 476 nm and highest in 514 nm. According to Purak and Noor (1990), the clumping of chromosomes may be attributed to the inhibition of protein synthesis.

In *A. cepa* the frequency of chromosome stickiness induced by lasers at 488 nm was higher than that induced by 476 and 514 nm. In *V. faba* the frequency of chromosome stickiness was higher at 488 and 514 nm than at 476 nm.

Stickiness and nondisjunction are due to disruption of bonds between protein and nucleic acid constituents or physical adhesion of proteinaceous matrix resulting in failure of chromosome condensation in prophase (Cohn, 1969; Stephen, 1979; Purak and Noor, 1990). According to Klasterka *et al*. (1976), stickiness is the result of the breaks and chromosome exchanges during prophase contraction. Apparently lasers affect the process of normal chromosome condensation.

Lasers also apparently cause chromosome breaks, as dicentric chromosomes, which are usually formed as a result of breaks in each arm of wo adjacent chromosomes and their reunion was the most commonly observed ype of stickiness. Multiple kinetochore chromosomes such as tricentric and quadricentric have been rarely observed, which apparently arise from breaks in nore than two chromosomes. The other type of stickiness observed rarely in aser irradiated samples were the chromosome rings. Inter arm asymmetric ype of chromosome interchanges are known to lead to centric ring of varying izes and a compound fragment. The rings may separate freely or open to a ingle dicentric ring or the two centric ring rings may be interlocked (Conger, .965). In very rare cases late condensation of chromosomes at metaphase were also observed in *V. faba*. Reciprocal translocations were observed at netaphase in both plant species. Symmetrical complete chromosome nterchanges lead to reciprocal translocations. According to Cohn (1969), these re difficult to detect unless chromosome morphology is drastically changed. Chromatid rings were very rarely observed at metaphase in both plant species. Two breaks within the same chromosome may lead to symmetrical or symmetrical interchange and the latter leads to chromatid ring (centric or centric) and a fragment. Double minutes were also observed very rarely in ome cases. Intra-arm asymmetrical complete type of intrachange leads to eleted piece in the form of an acentric ring or a pair of minutes. Double ninutes have no centromeres and regularly undergo nondisjunction at mitosis. Polyploid cells were also observed rarely at metaphase in both plant species. According to Darlington (1965), the spindle abnormality, polyploidy like irregular orientation and scattered distribution of chromosomes at metaphase re due to nonsynchronisation of division at centromore. Thus distribution of hromosomes is more confined to the periphery of the cell. Disturbed

metaphase due to spindle disturbance was also found in a few preparations. The chromosomes were found scattered without proper orientation at the metaphase plate.

The frequency of nondisjuction induced by laser irradiation of 514 and 488 nm was higher than that caused by 476 nm in both plant species.

The later stages of mitotic cell division are anaphase and telophase. The aberration commonly seen at anaphase were bridges and laggards and at telophase were micronucleate, binucleate and elongated nucleate cells.

In both plant species the frequency of bridges caused by laser exposures at 476 nm was higher than that induced by 488 and 514 nm. Bridges probably result from laser induced stickiness and bridge breakage fusion cycle (Cohn, 1969). In very rare cases star shaped anaphase was also observed.

Another type of aberration noticed at anaphase was laggards. Those chromosomes which are not taking part in bridge formation sometimes may get detached from the group and are seen lagging in the cell. This aberration was induced in very low frequency in laser treated samples. In both plant species, the frequency of laggards was higher in 476 nm laser treated samples than those exposed to 488 and 514 nm lasers.

One of the aberration observed at telophase was cells with micronuclei. In *A. cepa,* cells with micronuclei were seen in very low frequency in only some of the laser exposures. In *V. faba*, laser exposures of 514 and 488 nm induced micronucleate cell formation in higher frequencies than 476 nm.

Another common aberration seen in telophase is the formation of binucleate cells. In both plant species binucleate cells were more frequent in 514 and 488 nm than in 476 nm laser exposures. The formation of binucleate cells in laser treatments is attributed to the absence of cytokinesis (Eigsti and Dustin, 1957), which may be due to the inhibition of cell plate formation. The continuous fiber forms the spindle of cytokinesis upon which the cell plate formation occurs.

The presence of cells with highly elongated nuclei were another aberration seen at telophase. In both plant species the formation of elongated nucleate cells was several fold more frequent in samples exposed to 476 nm than 488 or 514 nm lasers. According to Walum *et al.*, (1990) changes in nucleic acid and protein synthesis changes cell volume or nucleus volume in mammalian cells.

In addition to these changes, high doses of treatments resulted in chromatin droplets in some cells and very rarely nuclear polymorphism and nuclear bridges were also encountered in some treatments. Eigsti and Dustin (1957) suggested the term pseudonuclei for chromatin dropletes resulted due to chromatin disintegration which remains scattered in the cytoplasm. Mercykutty and Stephen (1980) also observed such globular structures in *A. cepa* cells treated with high concentration of Adriamycin. According to them it is a unique type of chromosome aberration characterised by the transformation of chromatin into tiny globules and by the acute fragmentation of chromosomes.

5.2 Lasers of 488 nm wavelength at power density 400 mW induced higher mitotic aberration than lasers of 476 and 514 nm

The total mitotic aberration induced by laser exposures was maximum at 400 mW of 488 nm in both plant species. Differences in the cytogenetic and mutational effects of different wavelengths of laser irradiation has been reported by several workers. For instance, Vasiieva *et al*. (1990) reported differences in the mutation frequency and spectrum of changes induced by different doses of helium-neon lasers in the cvs. Auralia and Doukat. Similarly Akhmedova (1993) reported the dependence of mutation frequency *Gossypium* seeds on the irradiation intensity and Xu meifen (1991) reported differences in mutation frequency in wheat dependent on time of irradiation with CO_2 lasers.

5.3 Frequency of mitotic aberration induced by lasers is lesser than that caused by γ-irradiations but comparable to that induced by mutagens such as EMS and HA and higher than that caused by UV.

γ-irradiations produced metaphase chromosomal aberration, anaphase bridges and also high frequency of micronuclei in cells. The mutagenicity of γ-rays could be attributed to the high penetration power and ionisation power of γ-irradiations. UV rays on the other hand producing mitotic aberration at a very low frequency possibly because UV is a low energy radiation, does not cause ionisation and has a very limited penetrating capacity (one or two cells layers). Because of coherence and intensity laser exposures can penetrate deeply into tissues and this possibly accounts for the mitotic aberration frequency being higher than that caused by UV.

Prasad and Das (1980), Bhamburkar and Bhalla (1980), Reddy *et al* (1993) and Karpate (1996) have reported the different response of varieties to different mutagens. The type of mutagen, plant genotype and the physical state of the organism are considered to be important factors which contribute to the difference in frequency and spectrum of induced mutations. The difference in mode of action of mutagen (Okado *et al.*, 1972), differential penetration of the mutagen to the target (Kihlman, 1952), efficiency of repair process (Lawrence *et al*, 1974) and factors affecting the expression of concerned mutation (Auerbach, 1967) might also be playing a role in inducing mutations with varying frequency and spectrum. Sharma and Chatterjee (1962) and Varugheese and Swaminathan (1968) are of the opinion that mutagenic effectiveness and efficiency are due to the amount of DNA and its replication time in the initial stages. The physiological stage of the cell as well as the ability to repair the damage or several other physical factors (Brock, 1965; Chopra and Swaminathan, 1966; Auerbach, 1967; Gelin, 1968; Ilivea 1971).

5.4 The two plant species did not differ significantly in the spectrum or frequency of mitotic aberration caused by different mutagens.

Most of the mitotic aberration commonly encountered in mutagenic treatments were observed in various mutagen treatments in both plant species. However, in *A. cepa* γ-irradiations completely inhibited the growth of bulbs at an early stage of growth even at low doses (2kR). But in *V. faba,* it produced wide spectrum of aberration in all stages of mitotic cell division. The cells with micronuclei were produced in very high frequency in γ-irradiated *V. faba* seeds.

Effectiveness is a measure of gene mutation in relation to dose and efficiency is an estimate of biological effects, induced such as, lethality injury and sterility. Blixt (1968) is of the view that the sensitivity of ar organism depends upon the mutagen employed and genetic make up. The usefulness of any mutagen depends upon not only its effectiveness but also to a large extent upon its efficiency.

5.5 Lasers of 488 nm wavelength caused meiotic aberration more frequently than 476 and 514 nm

The mitotic aberration produced such as stickiness are more stable than other chromosomal aberration. Since 488 nm of laser exposure produced more aberration in the early stages of mitotic cell division the effect of it might have passed to the meiocytes and to the next generation.

Laser induced dose dependent increase in total meiotic aberrations with increase in power density in all the three wavelengths but the frequency of aberrations induced by 488 nm lasers was higher than that caused by 476 and 514 nm lasers. Xu Meifen (1991), conducted a similar type of study on the mutagenic effect of three lasers on L1 wheat, and reported a dose related variation in the mutagenic effects of the different lasers.

5.6 Lasers of 488 nm wavelength induced greater pollen sterility than other wavelengths tested

In laser exposures a dose dependent increase in pollen sterility with increase in treatment dose at 488 and 514 nm was observed while laser exposure at 476 nm induced decrease in pollen sterility. The frequency of

sterility induced by exposure of 30 min in power density 400 mW at 488 nm was highest.

400 mW power density of 488 nm induced highest mitotic as well as meiotic aberration. Usually the cells with aberration above a level may lead to death of the cell and finally the viability of the organism. As 488 nm of laser exposure showed more aberration in cells, it could be the reason for more pollen sterility.

According to Jana (1963), Cherry and Bhalla (1988), the pollen sterility increase with increase in mutagenic level. Gaul *et al.* (1966) and Ekberg (1969) suggested that sterility following mutagenic treatments might be attributed to detectable chromosomal aberration and cryptic deficiencies. The increase in pollen sterility was attributed by Reddy *et* al. (1992) to increase in cytological abnormalities Jana (1963) reported that the degree of chromosome pairing was mainly responsible for increased sterility in mutagenic population of black gram.

5.7 Laser irradiation produced fewer meiotic aberration than γ-rays, EMS and HA but was a stronger mutagen than UV

The commonly observed types of meiotic aberration were clumping and stickiness of chromosomes, bridges, laggards, unequal distribution of chromosomes and very rarely pollen with micronuclei.

Laser exposures induced dose dependent increase in total meiotic aberration with increase in power density in all three wavelengths. While UV exposures induced dose dependent decrease in the total aberration with increase in exposure time. γ-irradiations induced dose dependent increase in

induced by lasers. EMS treatment also induced dose dependent increase in this parameter with increase in concentration and was higher than that induced by laser exposures. Only a few pollen mother cells were observed in 1% EMS treatment but the aberration induced by it was several fold higher to that induced by laser exposures. HA treatment induced dose dependent decrease in total aberration and the values were similar to that induced by lasers.

An increase in bridges/fragments, laggards, abnormal cells and pollen sterility with increase in dose of gamma rays, duration of EMS, and concentration of sodium azide, either alone or in combination was reported by Reddy *et al.* (1992). Gamma ray treatments induced higher aberration than treatment with individual chemical mutagens. Similar results were also obtained in the work of Sharma and Sharma (1979) and Reddy and Annadurai (1992). Studies in various crop plants for instance, corn, (Beadle, 1930), rye (Prakken, 1943), lentil (Sinha,1967, Acharia and Sinha, 1975) also support this observation.

5.8 Lasers had a stimulatory effect on growth and yield in both plant species

Laser irradiations had a stimulatory effect on growth and yield in *A. cepa* and *V. faba*. γ-irradiations and HA treatments in higher doses/concentrations induced decrease in plant growth.

In *A. cepa,* growth rate induced by all three wavelengths of laser exposures were higher than control. Also, growth rate induced by 514 nm was higher than 488 nm. In *V. faba* growth rate induced by laser exposure of 476

nm was similar to control but the growth rate induced by 488 and 514 nm laser exposures was higher than control.

In *A. cepa,* UV exposures caused growth rate similar to control but in *V. faba* UV exposures induced growth rate higher than control. γ-irradiation in *V. faba* induced dose dependent decrease in growth rate with increase in irradiation dose. 2 and 5 kR γ-irradiated samples was similar to control. In *A. cepa,* γ-irradiations of bulbs inhibited the sprouting. EMS treatment induced growth rate lower than control in *A. cepa* but in *V. faba* EMS induced growth rate higher than control. Growth rate caused by 0.2% of EMS treatment was higher than that caused by 1% EMS treatment in both plant species.

In *A. cepa,* HA treatment caused growth rate higher than control and was similar to that induced by 488 514 nm of lasers but in *V. faba* HA treatments induced growth rate lower to control and that induced by laser exposures of 488 and 514 nm. Plants treated with 1% HA did not attain full growth.

Mutations affecting the plant height have been reported by several workers (Chen and Gottschalk, 1970; Oknno and Kawai, 1978; Raisinghami and Mahna, 1994; Prasad and Ramesh, 1996). The plant height in mutants was reported to be affected by internodal length and alternations in number of nodes by Weber and Gottschalk (1973) while Bloustein and Gale (1984) proposed that the internodal length was probably affected by cell number, cell length or both.

The stimulatory effect found in low doses of γ-irradiations and UV exposures could be caused by elevated auxin level (Gordon, 1957; Gowda, 1977). Several other workers have also found low doses of mutagens to have

stimulatory effect in different crops (Sparrow, 1966., Khanna, 1988). Krishna *et al.* (1984) reported such changes in Rhodes grass, Ashri and Herzog (1972) in peanut, Borika and Birajdar *et al.* (1983) in cowpea (*Vigna unguiculata*), Malani *et al.* (1994) in *Abelmoschus*. Plant height, chlorophyll content, free amino acids, proteins and nucleic acids affected by dose / concentration in groundnut was reported by Venkatachalam and Jayabalan (1995). Similar observations have been made in different crops by several workers for instance in rice. (Inoue *et al.,* 1975) in chickpea (Khanna 1991) and barley (Giacomelli *et al.,* 1967). The production of soluble proteins, nucleotides and RNA affected growth reduction by X-irradiation in corn and peanut seedlings (Van Huystee *et al.*, 1968). DNA and RNA contents affected by dose in cowpea was reported by Khanna (1991).

Reduced growth in mutagen treatments due to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances was noticed by several workers (Gordon, 1957; Gunkel and Sparrow, 1961; Singh, 1974; Usuf and Nair, 1974). Chromosome breakage during mitotic inhibition (Evans and Sparrow, 1961), and the effect of the mutagens on the metabolism of the organism (Jain and Khanna, 1987) could be the reason for retarded plant growth, sterility and death in higher doses of mutagen treatments.

Stimulatory effect of lasers on growth and yield have been reported by many workers in several crops such as small-fruit cultivar and Pobeda cucumbers (Cholakov, 1990), *Vigna radiata* seedlings (Govil *et al.* 1991), in winter wheat, spring barley and pea (Zubal, 1990), in alfalfa and a grass mixture (Nanova, 1991,1992), false acacia forms (*Robinia pseudoacacia* L.), (Batov and Kitin, 1993).

Radiations of wavelengths below 400 nm was known to affect plants and animals adversely. For instance sunflower and maize seedlings (Mark and Tevini, 1997), *Phaseolus vulgaris* (bush bean) (Sailemark *et al*. 1997) and bean (Antonelli *et al*. 1997).

Gamma rays are powerful mutagenic agent and attack macromolecules in cells directly. Effects of γ-rays on plant growth and metabolism has also been extensively studied. Like UV radiation, γ-rays has detrimental effects on plants. Induction of physiological damage by γ-rays were confirmed by the observations of Abraham and Desai, 1996; Pandey and Gaur, 1984; Sidel, 1988; Geir, 1989 and Gaul, 1977. Mutagenesis has been reported in several crops for instance in *Vigina unguiculata* (Subramanyam, 1983), in Kalaynsona and Arjun of wheat cultivars (Khamankar, 1983) and Okra variety 2-2 (Malani *et al.,* 1983).

5.9 Laser irradiation is similar to gamma irradiation and EMS treatment in that it causes a change in soluble protein content and enzyme activities

In *A. cepa* laser exposures in all the three wavelengths induced increase in soluble protein content compared to control. In *V. faba,* laser exposures of 476 and 488 nm induced increase but 514 nm induced decrease in soluble protein content in cotyledon while in embryo the soluble protein content showed variation when compared to control. In *A. cepa,* γ-irradiations induced dose dependent decrease in soluble protein content and was lower than control. In *V. faba,* γ-irradiations induced considerable variation in protein content on third day and sixth day in samples.

In *A. cepa* laser exposures in all the three wavelengths induced increase in soluble protein content than control. In *V. faba* the soluble protein content increase in cotyledon induced by laser exposures of wavelength 476 and 488 nm but the soluble protein content in the embryo showed decrease on third day than control, while on sixth it was near control values. In *A. cepa* γ-irradiations induced dose dependent decrease in soluble protein content and was lower than control. In *V. faba* γ-irradiations induced increase in soluble protein content in cotyledon and the effect of γ-irradiations was more than that of lasers at certain doses.

In *A. cepa* EMS treatment induced decrease in soluble protein content with increase in concentration and was lower to control value. In *V. faba* also EMS treatment caused decrease in soluble protein content in cotyledon on third day and increase in soluble protein content on sixth day when compared to control but in embryo soluble protein content showed variations on third day and sixth days.

According to Jain and Khanna (1987), mutagens affect the metabolism of the individuals and influence the activity or synthesis of enzyme and growth regulators. Kerepesi *et al.* (1992), reported the increase in soluble protein content by laser beams of wavelength 337.1 nm. Prasad and Varma (1983) isolated high protein mutants by gamma irradiation of french bean seeds.

In *A. cepa,* laser exposures at 476 and 488 nm induced no significant change in protease activity but 514 nm induced decrease in protease activity with increase in power density and exposures time. In *V. faba,* laser exposures of 476 and 488 nm induced stimulation in protease activity but 514

irradiations induced dose dependent decrease in protease activity. In *V. faba,* γ-irradiations induced stimulation in protease activity. In *A. cepa* EMS treatment caused no significant variation in protease activity compared to control. In *V. faba*, protease activity in EMS treated samples did not show much variation in embryo and cotyledon.

In *A. cepa,* laser exposures induced increase in amylase activity when compared to control. In *V. faba,* laser exposures of low power density stimulated amylase activity but laser exposures of high power density decreased amylase activity. In *A. cepa,* γ-irradiations induced increase in amylase activity compared to control. In *V. faba,* γ-irradiations stimulator amylase activity and was higher than control in all irradiations but dose dependent decrease in enzyme activity was seen. Enzyme activity change was higher in embryo than in cotyledon. In *A. cepa,* EMS treatment caused significant decrease in enzyme activity at 0.2% although much difference was noticed at 1% when compared to control. In *V. faba,* EMS treatment effect decrease in amylase activity in cotyledon and embryo on fourth day of treatment but on second day, variation in amylase activity was found when compared to control.

Irradiation by lasers or γ-rays and EMS treatments may possibly be perceived as a stress by the plant because of which a change in protein content and enzyme activity possibly may occur. Conditions like salt stress was also reported to increase amylase and protease activity at maximum salt concentration was reported by Singh and Singh (1992).

B.10 Stimulation in sprouting / germination by lasers is not correlated with the change in activity of enzymes such as amylase and protease

Noggle and Fritz (1983) suggested that the major food reserves in seeds are starch, protein, nucleic acids etc. These foods, during germination, are mobilised to provide the embryonic axis with amino acids, nucleotides, inositol, sucrose, fatty acids and some inorganic ions. Several proteinases, nucleases and lipases present in dry seeds as inactive form become activated on availability of water. Whereas α - amylase is synthesised after water inhibition.

According to Bidwell (1979), the seed reserves during germination begin to be metabolised and translocated towards the growing tip, some of them as immediate breakdown products of storage compounds and others after some further metabolism in storage tissues. Amylases and proteases are the key enzymes required for mobilisation of food reserves to initiate growth (Bidwell, 1979). Nourishment for the seedlings is provided through this process until it becomes capable of supporting itself on external nutrients. The seedlings subsist on carbon reserves in the endosperm or cotyledon until the first leaves are approaching maturity to begin carbon autotrophy. The process of sprouting of onion bulbs is essentially similar to the germination of seeds with the initial food reserves being provided by the outer fleshy scale leaves for the development of the inner true leaves.

In *A. cepa* and in *V. faba* laser exposures, γ-irradiations and EMS treatments induced activation or change in amylase and protease activity compared to control (untreated) samples; however only germination in laser irradiated samples was found to be stimulated. The superior sprouting/ germination indicates the involvement of other factors in such processes.

Certain environmental factors such as water, oxygen, temperature and light are known to affect sprouting/germination. Laser irradiation may cause localised increase in temperature or the alteration in light requirements due to which enhanced sprouting/germination occurs.

Presowing irradiation of *Lepidium sativum* seeds was reported to stimulate growth rate of seedlings, the fluorescence intensity of CTC-Ca^{2+} complex and to enhance pH levels in cells by 0.2 - 0.8 units (Skvarko and Demkiv, 1994). It was proposed that laser biostimulation of the growth process in *Lepidium sativum* seeds proceeded with the involvement of phytochrome red far-red reactions. Stimulation of seed germination and growth of sprouts and roots under condition of low dose oxidative stress induced by ionizing radiation and hydrogen peroxide was reported by Korystor and Narimanov (1997).

1 Treatment by laser exposures, gamma rays and EMS did not cause an increase in peroxidase and catalase activities

Superoxide anion radical (O^{2-}) produced by photo reduction of molecular oxygen react with numerous cell components, thereby causing inactivation of enzymes, pigment bleaching, lipid peroxidation and protein breakdown.

According to Srivastava and Strasser (1997), the key enzymes involved in removal of these superoxide radicals are superoxide dismutase, peroxidase and catalase, which prevent photoxidative damage. Magnesium and potassium deficiency and certain stress conditions like drought increase the activity of superoxide scavenging enzymes (Catmak and Ismail 1994; Mittler *et al*. 1994). The activity of these enzymes decreased in zinc deficient plants (Catmak and Marschner 1993).

In *A. cepa,* laser exposures induced no significant difference ir peroxidase and catalase activities in treated samples from control samples. Ir *V. faba,* laser exposures caused decrease in peroxidase and catalase activity. Ir *A. cepa,* γ-irradiations did not cause significant difference in peroxidase anc catalase activities in irradiated samples compared to control. In *V. faba,* peroxidase and catalase activity decreased in γ-irradiated samples except foı one or two doses when compared to control. EMS treatment in *A. cepa* caused increase in peroxidase activity compared to control. Catalase activity on second day increased but on fourth day no significant change in activity in EMS treated samples was noticed compared to control in inner true leaves in *A. cepa..* In *V. faba,* EMS treatment caused significant variation in peroxidase and catalase activities in treated samples compared to control. Peroxidase and catalase activities decreased in most of the treatments in *V. faba,* and in *A. cepa* the activity of these enzymes were negligible or absent in the outer fleshy scale leaves of onion bulbs and in embryo in *V. faba* indicating tissue specific variation for the enzyme.

Mutagenic effect of laser exposures, γ-irradiations and EMS treatments are indicated by several studies. It is tempting to speculate that a decrease in peroxidase and catalase activities may be the reason for increased chromosomal aberrations, as the accumulation of superoxide radical may alter macromolecules. However further studies in this area are required in order to identify the molecular basis of the mutagenecity of lasers and other mutagens.

CONCLUSIONS

6.1 Mitotic chromosomal aberration

6.1.1 Prophase aberration

1. In *A. cepa* prophase aberration induced by laser exposures at 514 nm was maximum.
2. Prophase aberration induced by UV and Hydroxyl amine were lower than 514 nm and similar to 476 nm and 488 nm of laser exposures but the prophase aberration induced by EMS treatment was higher than 476 nm and 488 nm and lower than 514 nm of laser exposures.
3. In *V. faba* prophase aberration induced by 488 nm and 514 nm were higher and similar and higher than that induced by 476 nm.
4. UV, γ-irradiations and EMS treatments induced prophase aberration lower than 488 nm and 514 nm but similar to 476 nm laser exposures. HA induced prophase aberration higher than 476 nm and lower than 488 and 514 nm laser exposures.

6.1.2 Metaphase aberration

1. In *A. cepa* metaphase aberration induced by 488 nm and 514 nm of laser exposures were similar.
2. Metaphase aberration caused by UV was lower than 488 and 514 nm but similar to 476 nm of laser exposures. Metaphase aberrations induced by EMS and HA treatments were similar and was higher than that induced by laser exposures.
3. In *V. faba* metaphase aberration induced by 488 nm of laser exposure was maximum.

4. Metaphase aberration induced by γ, EMS and HA treatments were higher than that caused by all three wavelengths of laser exposures. UV induced metaphase aberration frequency similar to 488 nm and higher than 476 nm and 514 nm.

5. In *A. cepa* anaphase aberration induced by laser exposures were maximum at 476 and 488 nm and were similar in frequency.
6. Anaphase aberration caused by UV and EMS were similar to that induced by 476 and 488 nm but higher than 514 nm laser exposures. HA induced anaphase aberration similar to 514 nm but lower than 476 and 488 nm lasers.
7. In *V. faba,* anaphase aberration induced by 476 nm of laser exposure was maximum.
8. Anaphase aberration induced by γ-irradiations and that induced by 476 nm of laser exposure were similar but higher than 488 and 514 nm of laser exposures, while the anaphase aberration induced by UV was lower than that induced by all laser exposures. The frequency of anaphase aberrations induced by EMS and HA treatments were lower to 476 nm but similar to 488 nm and 514 nm of laser exposures.

6.1.3 Telophase aberration

1. In *A. cepa,* telophase aberration induced by laser exposures were similar and maximum at 476 and 488 nm of laser exposures.
2. Telophase aberration induced by UV and HA treatments were lower than that induced by laser exposures of all three wavelengths but telophase aberration induced by EMS was similar to that induced by 514 nm and lower than 476 and 488 nm of laser exposures.

3. In *V. faba,* laser exposure of 488 nm induced maximum telophas aberration frequency.
4. Telophase aberration induced by γ-irradiations were several fold higher tha that induced by laser exposures. Telophase aberration induced by U exposures was similar to that induced by 514 nm but lower than 476 an 488 nm of laser exposures. EMS caused telophase aberration similar to 47 nm, higher to 514 nm and lower than 488 nm of lasers. HA treatmer induced telophase aberration similar to that induced by 514 nm but lowe than 476 and 488 nm of laser exposures.

6.1.4 Total mitotic aberration

1. In *A. cepa,* total mitotic aberration was maximum at 400 mW power densit of 488 nm and 514 nm laser exposures.
2. Total mitotic aberration induced by UV exposures was lower than tha caused by laser exposures in all three wavelengths. Total aberration induce by EMS treatment was similar to that induced by 400 mW power density c 488 and 476 nm but higher than 514 nm and 200 mW of 488 nm and 47 nm Total aberration induced by HA treatment was similar to that induced b 514 nm and 200 mW of 476 nm but lower to 488 nm and 400 mW of 51 nm laser exposures.
3. In *V. faba,* total mitotic aberration was maximum at 400 mW power densit of 488 nm laser exposure.
4. Total aberration induced by UV, EMS and HA were similar to that caused b 514, 476 nm and 200 mW of 488 nm but lower than 400 mW of 488 nn laser exposures. Total aberration induced by γ-irradiations were 4-5 fol higher to that induced by laser exposures.

6.1.5 Mitotic index

1. In *A. cepa,* laser exposures induced lowest mitotic index at 488 nm.
2. Mitotic index induced by UV exposures were higher than 488 nm but similar to 476 and 514 nm laser exposures. Mitotic indices induced by EMS and HA treatments were similar to that induced by laser exposures of 514 nm but higher than 488 nm and lower than 476 nm.
3. In *V. faba,* laser exposures induced mitotic indices similar in all three wavelengths.
4. Mitotic index induced by UV exposures was higher than that induced by laser exposures. Mitotic indices of EMS, HA and γ-irradiations were similar to the mitotic indices induced by laser exposures.

6.2 Meiotic aberration

6.2.1 Clumping of chromosomes

1. Clumping of chromosomes induced by laser exposures was maximum at 488 nm.
2. Clumping of chromosomes induced by γ-irradiations and EMS treatments were higher than that induced by laser exposures in all three wavelengths. Clumping of chromosomes induced by UV and HA treatments was higher than that induced by 476 and 514 nm but lower than 488 nm of laser exposures.

Stickiness of chromosomes

3. Stickiness in laser treatments was maximum at 514 nm.
4. Stickiness induced by UV exposures was similar to that induced by 476 and 488 nm of laser exposures but lower than 514 nm of laser exposure. Stickiness induced by γ and EMS treatments was higher than that induced

by laser exposures. Stickiness induced by HA treatment was similar to 51[illegible] nm but higher than 476 and 488 nm of laser exposures.

6.2.2 Bridges

1. Bridges induced by laser exposures was highest at 200 mW power density of 514 nm.
2. Bridges were absent in UV exposures. Bridges induced by γ-irradiations were similar to that induced by laser exposures but bridges induced by EMS 0.2% was similar and EMS 1% was several fold higher than that induced by laser exposures. Bridges induced by HA treatment was higher than that induced by 476 nm of laser exposure and 200 mW power density of 488 nm, similar to 400 mW power density of 514 nm and lower than 400 mW of power density of 488 nm and 200 mW power density of 514 nm.

6.2.3 Laggards

1. Laggards were not found in all laser exposures. 488 nm of laser exposure induced highest laggard frequency.
2. Laggards were not induced by UV exposures, 1% concentration of EMS and 0.2% of concentration of HA. Laggards induced by γ-irradiations were similar to that induced by laser exposures while EMS and HA treatments induced laggard frequency lower than that induced by laser.

6.2.4 Unequal distribution of chromosomes

1. Unequal distribution of chromosomes in laser exposures were maximum at 200 mW power density of 514 nm.
2. Unequal distribution of chromosomes induced by UV exposures and HA treatments was higher than that induced by laser exposures but the same induced by γ-irradiations was lower than that induced by laser exposures.

Unequal distribution of chromosomes induced by EMS treatments were several fold higher than that induced by laser exposures.

6.2.5 Micronucleate cells

1. Micronucleate cells were not found in most of the treatments. It was seen only at 200 mW power density of 488 nm. Micronucleate cells induced by UV 3 hour, 15 KR γ-irradiation and HA 1% treatments were higher than that induced by laser exposures.

6.2.6 Total meiotic aberration

1. Total meiotic aberration was found to be highest at 488 and 514 nm of laser exposure.
2. Meiotic aberration induced by UV has lower than that induced by 488 nm and 514 nm and higher than 476 nm laser exposures but γ-irradiations and EMS treatments caused increase in total aberration compared to that induced by laser exposures. HA caused total aberration similar to that induced by laser exposures.

6.3 Pollen sterility

1. Pollen sterility was maximum at 400 mW power density of 488 mW.
2. Pollen sterility induced by UV exposures and 2,5,10 and 15 kR's of γ-irradiations, 0.2% EMS and HA treatments were similar to that induced by 476 nm and 200 mW power density of 488 nm but lower than 400 mW power density of 488 nm and 514 nm laser exposures. Pollen sterility induced by 20 kR of γ-irradiations, 1% of EMS treatments and 1% HA treatments were several fold higher than that induced by laser exposures.

6.4.1 Sprouting/germination index

1. Sprouting index in *A. cepa* induced by 476 nm and 200 mW power densit of 514 nm was maximum in laser exposures. Sprouting index of 476 nr was similar to control value but 200 mW power density of 514 nm wa higher to control value.
2. Sprouting index induced by UV 3 hour and EMS 1% treatments was simila to these and higher than that induced by 488 nm and 400 mW powe density of 514 nm laser exposures and was higher than control value Sprouting index of EMS 0.2% and HA 1% treatments were lower than tha induced by laser exposures and control value. Sprouting index induced b UV 1 hour and HA 0.2% were similar to control and that induced by 400 mV power density of 488 nm and 514 nm but 200 mW power density of 488 nn was lower than it.

3. Germination index in *V. faba* induced by 488 nm laser exposure wa maximum.
4. Germination index of 2 kR and 5 kRs's of γ-irradiations was similar to contrc value and was higher than that induced by laser exposures. But 10 kR, 1! kR and 20 kR induced dose dependent decrease in germination index an(was several fold lower than control and that induced by laser exposures. U\ induced germination index similar to that induced by 514 nm lase exposures but lower than that induced by 488 nm laser exposure an(control. EMS induced germination index higher than that induced by lase exposures. This value of 0.2% EMS treatment was higher and of 1% simila to that of control value. HA treatment induced germination index severa fold lower than that induced by laser exposures and control.

6.4.2 Growth rate

1 In *A. cepa,* growth rate induced by laser exposures was maximum at 514 nm and was higher to control.

2 The growth rate induced by UV 1 hour was higher than control, similar to that induced by 488 nm of laser exposure but lower than 514 nm of laser exposure while UV 3 hours induced growth rate lower than control and that induced by laser exposure.

3 EMS 0.2% induced growth rate similar to but 1% lower than control. Growth rate induced by EMS was lower than that induced by laser exposures. HA treatment induced growth rate higher to control but similar to 488 nm and lower than 514 nm laser exposures.

4 In *V. faba,* growth rate was maximum at 400 mW of 488 nm induced by laser exposure and higher than that of control.

5. UV induced growth rate lower than that induced by laser exposures and was higher than control. γ-irradiations induced growth rate lower than that induced by laser exposures. 2 kR and 5 kR growth rate was similar but 10 kR, 15 kR and 20 kR lower than that of control. EMS treatments induced growth rate lower than that induced by laser exposures but higher than that of control (untreated) value. HA treatment induced decrease in growth rate and was lower than that induced by laser exposures and control. 1% HA treatment plants di not attained full growth.

6.4.3 Yield

1. In *A. cepa,* yield induced by laser exposures was maximum at 400 mW power density of 488 nm and was almost two fold higher than the yield of control.

2. UV exposures, EMS and HA treatments induced yield higher than that of control but lower than the yield induced by laser exposures.
3. In *V. faba* yield was maximum at 400 mW power density of 488 nm and the yield in laser exposures was higher than the yield of control.
4. Yield in UV 1 hour exposure were similar than the yield of 514 nm and 200 mW power density of 488 nm but lower than 400 mW power density of 488 nm laser exposures and similar to the yield of control. UV 3 hours exposure induced yield was lower than all laser exposures and control. Yield in γ-irradiated, EMS treated and HA treated samples was lower than control and laser exposures yield value. γ-showed dose dependent decrease in yield and 1% HA treated plants did not survive till maturity.

6.5.1. Soluble protein content

1. In *A. cepa,* laser exposures induced increase in soluble protein content in inner true leaves and outer fleshy scale leaves higher than control. Soluble protein content in outer fleshy scale leaves was higher than in inner true leaves.
2. γ-irradiations induced decrease in soluble protein content in inner true leaves and outer fleshy leaves lower than control. EMS treatments also induced decrease in soluble protein content in inner true leaves and outer fleshy scale leaves and it was lower than the control.
3. In *V. faba,* laser exposures of 476 nm and 488 nm induced increase in soluble protein content but 514 nm decreased soluble protein content when compared to control. Soluble protein content in embryo was less than control on third day but similar to control on sixth day. Soluble protein content in cotyledon was higher than that of the embryo in control, 476 and

488 nm of laser exposures but at 514 nm of laser exposures soluble protein content in embryo was higher than that in cotyledon.

4. γ-irradiations of all doses induced increase in soluble protein content on sixth day in cotyledon than control but on third day it was similar to control. In embryo on third day soluble protein content in γ-irradiated samples was less than control but on sixth day it was similar to control.
5. EMS treatments caused decrease in soluble protein content in cotyledon on third and increase in soluble protein content on sixth day when compared to control. Soluble protein content in embryo decreased on sixth day and on third day decreased at 0.2% and significantly increased at 1% EMS treatment when compared to control. Soluble protein content in cotyledon was higher than in embryo.

6.5.2 Protease activity

1. In *A. cepa,* laser exposures at 476 and 488 nm induced no significant change in activity compared to control but specific activity increased with increase in dose in samples exposed to these two wavelengths. Activity and specific activity was higher than outer fleshy scale leaves than inner true leaves. Activity and specific activity decreased with increase in power density at 514 nm of laser exposures.
2. γ-irradiations induced decrease in activity and specific activity of protease in *A. cepa*. EMS treatment did not show significant variation in treated samples compared to that of control. Specific activity in inner true leaves of samples treated with EMS showed decrease with increase in dose on second day and was several fold lower than control, but specific activity in outer fleshy scale leaves on second day showed increase with increase in dose and was higher than control. Significant variation was not observed on fourth day in specific activity in outer fleshy scale leaves and in inner true leaves.

3. In *V. faba,* laser exposures at 476 and 488 nm induced increase in activity and specific activity in cotyledons and embryo and was higher than control value. ncrease in activity with increase in power density was found at 488 nm but decrease in enzyme activity with increase in power density was found at 476 nm. 514 nm caused decrease in enzyme activity. Variation in specific activity was found in embryo but no variation in specific activity was found in cotyledon.
4. -irradiations induced no significant change in enzyme activity and specific activity. Activity in treated samples was higher than control. EMS treatment did not show much variation in enzyme activity in embryo and cotyledon. It was lower than control in embryo and similar to control in cotyledon specific activity showed variation in embryo with control but no significant variation was observed in cotyledon.

6.5.3 Amylase activity

1. In *A. cepa* laser exposures did not show significant change in amylase activity compared to control but specific activity was higher than control.
2. γ-irradiations caused dose dependent increase in activity and specific activity. EMS treatment caused significant decrease in enzyme activity at 0.2% although not much difference was noticed at 1% when compared to control. Specific activity showed variation in inner true leaves and outer fleshy leaves when compared to control.
3. In *V. faba,* no significant difference in amylase activity in laser exposed samples compared to control samples was observed. Specific activity in embryo showed variation but cotyledon showed no significant variation compared to control.
4. γ-irradiations induced slightly higher activity and specific activity in cotyledon and embryo compared to control. EMS treatment caused decrease

in amylase activity in cotyledon and embryo on fourth day but on second day variation in activity was found when compared to control. Specific activity showed no significant change in cotyledon but in embryo variation was observed compared to control.

6.5.4 Peroxidase activity

1. In *A. cepa,* peroxidase activity is significantly absent or very low in outer fleshy scale leaves as compared to the inner true leaves. Laser exposures caused no significant change in enzyme activity in inner true leaves compared to control.
2. γ-irradiations caused transient increase in peroxidase activity in irradiated samples compared to control and no dose dependence was found. EMS treatment also caused an increase in peroxidase in inner true leaves compared to control.
3. In *V. faba,* laser exposure at 514 nm induced increase in peroxidase activity in cotyledon. Peroxidase activity was lower than control in the cotyledon and embryo in all other laser exposures.
4. γ-irradiations showed variations in peroxidase activity in embryo and cotyledon in *V. faba* compared to control. EMS caused transient increase in peroxidase activity in embryo with increase in treatment concentration but in cotyledon enzyme activity showed variation compared to control.

6.5.5 Catalase activity

1. In *A. cepa,* catalase activity was significantly absent in the outer fleshy leaves compared to the inner true leaves. Laser exposures at 488 nm and 514 nm caused no significant change in catalase activity but 476 nm caused increase in activity with dose.
2. γ-irradiations induced increase in enzyme activity in irradiated samples compared to control but no dose dependence was found. EMS treatment

caused increase in catalase activity on second day compared to control ir inner true leaves but on fourth day variation in activity was found.

3. In *V. faba,* catalase activity was absent in embryo and found only ir cotyledon of both control and treated samples.
4. Laser exposures of 476 nm and 514 nm decreased enzyme activity with increase in power density but at 488 nm the activity increased with increase in power density in *V. faba*.
5. γ-irradiations induced decrease in enzyme activity compared to control and varied in different doses. EMS treatment caused variation in enzyme activity in embryo in *V. faba*.

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2. P.R. Unnikrishna Pillai, Padma Nambisan, V.P.N. Nampoori and C.P.G. Vallabhan. Induction of Mitotic Aberrations by Argon Ion Lasers in *Vicia faba* and *Allium cepa L.* ***Genetics and Breeding*** (Bulgaria). 1998. (In press).

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App. I A

Effect of Argon ion laser at 476 nm at 200 and 400 mW on mitosis in *A. cepa*

Abnormalities (% of total cells)	Treatment		Time 10 min	20 min	30 min	40 min
Clumping	Control	0.03				
	200		0.047	0.06	0.07	0.07
	400		0.069	0.073	0.07	0.10
Stickiness	Control	0.00				
	200		0.085	0.13	0.148	0.13
	400		0.127	0.126	0.933	0.138
Nondisjunction	Control	0.09				
	200		0.12	0.195	0.166	0.142
	400		0.118	0.147	0.149	0.175
Bridges	Control	0.00				
	200		0.194	0.145	0.157	0.259
	400		0.255	0.157	0.18	0.22
Laggards	Control	0.00				
	200		0.054	0.014	0.026	0.03
	400		0.049	0.08	0.093	0.4
Micronucleate	Control	0.06				
	200		0.00	0.00	0.00	0.00
	400		0.00	0.00	0.00	0.018
Binucleate	Control	0.05				
	200		0.00	0.00	0.00	0.018
	400		0.00	0.01	0.031	0.276
Elongated nucleate	Control	0.00				
	200		0.99	0.64	0.864	1.48
	400		0.28	1.65	1.00	2.9
Total aberrations (%)	Control	0.17				
	200		1.49	1.097	1.431	2.13
	400		0.899	2.243	1.65	3.75
Total No. of cells observed	Control	6472				
	200		12862	11677	11456	11191
	400		10204	9526	12793	1053
Mitotic index	Control	4.74				
	200		3.28	2.93	2.88	2.94
	400		3.16	2.95	2.77	1.94

App. II A

Effect of Argon ion laser at 488 nm at 200 and 400 mW on mitosis in *A. cepa*

Abnormalities (% of total cells)	Treatment		Time 10 min	20 min	30 min	40 min
	Control	0.03				
	200		0.10	0.05	0.10	0.06
Clumping	400		0.06	0.098	0.05	0.052
	Control	0.00				
	200		0.224	0.31	0.21	0.20
Stickiness	400		0.138	0.098	0.087	0.17
	Control	0.09				
	200		0.317	0.22	0.19	0.26
Nondisjunction	400		0.24	0.39	0.25	0.23
	Control	0.09				
	200		0.317	0.22	0.19	0.26
Bridges	400		0.24	0.39	0.24	0.23
	Control					
	200		0.28	0.018	0.034	0.040
Laggards			0.049	0.036	0.094	0.098
	Control	0.00				
	200		0.028	0.00	0.009	0.00
Micronucleate	400		0.00	0.00	0.00	0.00
	Control	0.05				
	200		0.214	0.35	0.34	0.21
Binucleate	400		0.00	0.214	0.23	1.68
	Control	0.00				
	200		0.71	0.60	1.31	0.84
Elongated nucleate	400		0.74	1.56	0.94	0.17
	Control	0.17				
	200		1.64	1.56	2.22	1.67
Total aberrations (%)	400		1.48	2.46	2.66	2.42
	Control	6472				
Total No. of	200		10709	10946	11569	12240
cells observed	400		12321	11209	13788	15424
	Control	4.74				
	200		2.28	2.09	2.05	2.068
Mitotic index	400		2.45	1.92	1.79	1.73

App. III A

Effect of Argon ion laser at 514 nm at 200, 400, 600 and 800 mW on mitosis in *A. cepa*

Abnormalities (% of total cells)	Treatment		Time 10 min	20 min	30 min	40 min
Clumping	Control	0.03				
	200		0.56	0.42	0.37	0.47
	400		0.39	0.48	0.43	0.38
	600		0.33	0.25	0.30	0.20
	800		0.32	0.36	0.28	0.32
Stickiness	Control	0.00				
	200		0.04	0.03	0.06	0.12
	400		0.06	0.08	0.05	0.18
	600		0.02	0.02	0.01	0.00
	800		0.09	0.06	0.06	0.02
Nondisjuction	Control	0.09				
	200		0.38	0.49	0.33	0.23
	400		0.41	0.08	0.27	0.41
	600		0.03	0.14	0.11	0.12
	800		0.18	0.12	0.09	0.23
Bridges	Control	0.00				
	200		0.06	0.04	0.14	0.12
	400		0.10	0.10	0.03	0.03
	600		0.08	0.13	0.14	0.18
	800		0.18	0.12	0.09	0.23
Laggards	Control	0.00				
	200		0.02	0.018	0.058	0.033
	400		0.06	0.06	0.016	0.05
	600		0.030	0.032	0.043	0.066
	800		0.048	0.016	0.073	0.0376
Micronucleate	Control	0.00				
	200		0.04	0.02	0.00	0.07
	400		0.00	0.00	0.00	0.00
	600		0.00	0.00	0.00	0.00
	800		0.02	0.00	0.00	0.00
Binucleate	Control	0.05				
	200		0.16	0.20	0.45	0.43
	400		0.80	0.12	0.54	0.10
	600		0.46	0.59	0.64	0.67
	800		0.51	0.59	0.57	0.49
Elongated nucleate	Control	0.00				
	200		0.26	0.13	0.27	0.23
	400		0.33	0.30	0.02	0.20
	600		0.17	0.06	0.13	0.05
	800		0.03	0.15	0.22	0.30
Total aberrations (%)	Control	0.17				
	200		1.52	1.368	1.678	1.703
	400		2.108	1.52	1.356	1.31
	600		1.12	1.22	1.393	1.34
	800		1.26	1.586	1.463	1.48
Total No. of cells observed	Control	6472				
	200		5007	5470	5148	5988
	400		4904	5014	6339	3926
	600		6586	6294	7011	6589
	800		6279	5217	5449	5313
Mitotic index	Control	4.74				
	200		3.44	2.91	2.43	2.35
	400		3.00	2.73	2.62	2.03
	600		1.94	1.73	1.63	1.37
	800		1.56	1.51	1.41	1.32

App. IV A

Effect of UV exposure at 1,2, and 3 hr on mitosis in *A. cepa*

Abnormalities (% of total cells)	Treatment			
Abnormalities (% of total cells)	Control	UV 1hr	UV 2hr	UV 3 hr
Clumping	0.03	0.036	0.55	0.089
Stickiness	0.00	0.042	0.041	0.04
Nondisjunction	0.09	0.096	0.123	0.19
Bridges	0.00	0.295	0.13	0.18
Laggards	0.00	0.10	0.001	0.102
Micronucleate	0.00	0.006	0.02	0.00
Binucleate	0.051	0.042	0.034	0.027
Elongated nucleate	0.00	0.61	0.226	1.02
Total aberrations (%)	0.17	1.23	1.075	1.652
Total No. of cells observed	6472	6626	7361	7846
Mitotic Index	4.74	2.89	2.21	3.22

App. V A

Effect of EMS at 0.2, 0.4, 0.6, 0.8 and 1% concentrations (30 min) on mitosis in *A. cepa*

Abnormalities (% of total cells)	Treatment					
Abnormalities (% of total cells)	Control	0.2%	0.4%	0.6%	0.8%	1%
Clumping	0.03	0.105	0.13	0.12	0.17	10.21
Stickiness	0.00	0.191	0.173	0.225	0.323	0.35
Nondisjunction	0.09	0.342	0.356	0.57	0.395	0.43
Bridges	0.00	0.065	0.11	0.17	0.20	0.18
Laggards	0.00	0.05	0.087	0.107	0.15	0.153
Micronucleate	0.00	0.00	0.00	0.00	0.01	0.00
Binucleate	0.05	0.053	0.18	0.085	0.14	0.16
Elongated nucleate	0.00	0.77	1.139	0.81	0.923	0.993
Total aberrations (%)	0.17	1.582	2.175	2.057	2.311	2.476
Total No. of cells observed	6472	7599	9221	9336	8354	9973
Mitotic index	4.74	2.95	2.66	2.12	1.91	1.64

App. VI A

Effect of HA at 0.2, 0.4, 0.6, 0.8 and 1% concentration (1 hr) on mitosis in *A. cepa*

	Treatment					
Abnormalities (% of total cells)	Control	0.2%	0.4%	0.6%	0.8%	1%
Clumping	0.03	0.08	0.072	0.129	0.09	0.09
Stickiness	0.00	0.20	0.22	0.249	0.147	0.26
Nondisjunction	0.09	0.27	0.31	0.429	0.56	0.69
Bridges	0.00	0.05	0.093	0.049	0.07	0.07
Laggards	0.00	0.03	0.08	0.039	0.039	0.049
Micronucleate	0.00	0.00	0.00	0.00	0.00	0.00
Binucleate	0.05	0.10	0.06	0.16	0.14	0.28
Elongated nucleate	0.00	0.30	0.62	0.52	0.509	0.36
Total aberrations (%)	0.17	1.03	1.455	1.575	1.55	1.8
Total No. of cells observed	6472	9860	9684	10020	10200	10182
Mitotic index	4.74	2.98	2.3	2.115	1.75	1.59

App. VII A

Effect of mutagens on sprouting index, growth and yield in *A. cepa*

Sl. No.	Treatment	Sprouting index	Plant height (cm)				Yield
			Days				Bulb weight (g)
			20	40	60	80	
1.	Control	100	16.6	22.8	23.45		
2.	476 nm 200mW -10′	100	15.5	17	25.5		
3.	476 nm 400mW -30′	100	16.9	17.71	23.94		
4.	Control	95	12.89	14.17	20.72	27.82	25.66
5.	488 nm 200mW-10′	80	13.19	13.25	19.87	28.75	25.62
6.	488 nm 400mW-30′	95	12.52	14	19.61	30.12	47.01
7.	514nm 400mW-10′	100	14.25	15.15	21.25	29.5	40.8
8.	514nm 400mW-30′	95	15.58	15.42	22.26	32.42	44.36
9.	Control	90	12.67	23.28	23.06	27.94	12.85
10.	UV 1hr	95	15.42	20.79	26.67	29.55	16.70
11.	UV 3hr	100	15.1	20.5	20.28	26.52	12.68
12.	EMS (0.2%)	75	19.33	22.12	23.94	28.29	17.05
13.	EMS (1%)	100	17.79	22.2	20.10	26.29	14.58
14.	HA (0.2%)	95	16.68	21.23	21.41	30.4	16.94
15.	HA (1%)	65	11.69	20.73	22.54	29.27	18

App. VIIIA

Effect of laser, gamma and EMS treatments on soluble protein content in inner true leaves and outer fleshy scale leaves on second and fourth day of sprouting in *A. cepa*

Treatment	Inner true leaves		Outer fleshy scale leaves	
	Day			
	2	4	2	4
	Soluble protein (µg/ml)	Soluble protein (µg/ml)	Soluble protein (µg/ml)	Soluble protein (µg/ml)
Laser				
Control	1640	1550	1490	1740
476 nm				
200 mW -10′	2870	3505	1680	2565
400 mW -30′	1790	1875	1770	2145
488 nm				
200 mW -10′	1695	2280	1790	2400
400 mW -30′	1735	1415	1935	2350
514 nm				
200 mW -10′	1415	1305	1085	1745
400 mW -30′	1720	1225	1380	1895
Gamma				
2 kR	1145	-	1100	-
5 kR	1145	-	1195	-
10 kR	1260	-	1285	-
15 kR	1280	-	975	-
EMS				
Control	789.7	1031.6	2569.5	1422.4
0.2%	2540.4	1048.9	2029.4	1160.9
1%	2261.7	954.02	1747.8	1158

App. IX A

Effect of laser, gamma and EMS treatments on protease activity and specific activity in inner true leaves and outer fleshy scale leaves on second and fourth day of sprouting in *A. cepa*

Treatment	Inner true leaves				Outer fleshy scale leaves			
	Day							
	2		4		2		4	
	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity
Laser								
Control	0.476	1.2	0.606	1.87	0.85	2.05	0.751	1.84
476 nm								
200 mW -10′	0.702	1.224	0.574	0.82	1.01	3.01	0.797	1.55
400 mW -30′	0.563	1.57	0.326	0.87	1.054	2.97	0.916	2.14
488 nm								
200 mW -10′	0.66	1.94	0.614	1.34	1.13	3.16	0.728	1.5
400 mW -30′	0.643	1.85	0.47	1.6	1.06	2.74	0.91	1.9
Control	0.633	1.92	0.614	1.98	1.13	3.79	1.74	4.92
514 nm								
200 mW -10′	0.487	1.72	0.317	1.22	0.788	3.63	0.783	2.24
400 mW -30′	0.373	1.08	0.305	1.24	0.57	2.06	0.648	1.71
Gamma								
Control	0.633	1.92	0.614	1.98	1.13	3.79	1.74	4.92
2 kR	0.229	1	-	-	0.529	2.403	-	-
5 kR	0.229	1	-	-	0.409	1.711	-	-
10 kR	0.262	1.019	-	-	0.365	1.419	-	-
15 kR	0.218	0.825	-	-	0.234	1.202	-	-
EMS								
Control	0.84	5.32	0.383	1.86	1.142	2.226	0.541	1.905
0.2%	0.97	1.91	0.445	2.12	0.935	2.35	0.364	1.57
1%	0.65	1.44	0.445	2.12	1.176	3.36	0.46	1.98

App. X A

Effect of laser, gamma and EMS treatments on amylase activity and specific activity in inner true leaves and outer fleshy scale leaves on second and fourth day of sprouting in *A. cepa*

Treatment	Inner true leaves				Outer fleshy scale leaves			
	Day							
	2		4		2		4	
	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity
Laser								
Control	42.55	109.1	68.1	136.16	54.46	124.8	49.66	105.73
476 nm								
200 mW -10′	56.28	180.38	43.19	110	60.67	248.6	62.9	144.7
400 mW -30′	56.86	162.45	48.86	147.72	49.2	184.9	48.89	114.83
488 nm								
200 mW -10′	53.35	180	31.13	83.23	60.38	146	52.4	120.68
400 mW -30′	55.43	210.76	34.34	95.38	49.24	147.86	46.38	108.69
Control	49.3	133.9	34.67	188.32	52.3	229.3	41.38	175
514 nm								
200 mW -10′	43.34	182.21	30.17	269.9	50.51	251.1	29.05	105.48
400 mW -30′	48.81	166.7	29.8	278.6	60	372.6	35.1	97.7
Gamma								
Control	49.3	133.9	34.67	188.32	52.3	229.3	41.38	175
2 kR	58.2	411.3	45.76	184.57	59.1	371.69	61.89	223.4
5 kR	59.83	431.9	52.8	226.3	59.95	382.4	61.13	254
10 kR	48.09	440	52.5	423	48.76	337.7	60.42	404.2
15 kR	38.71	255.8	55.14	343.7	46.41	481	51.04	291
20 kR	32.68	194.8	56.02	327.6	50.1	429.3	52.21	286.2
EMS								
Control	50.25	217.53	29.03	159.95	64.93	838.02	51.01	114.2
0.2%	5.58	20.67	36.07	212.23	79.93	529.34	50.86	93.15
1%	53.19	199.21	46.37	219.76	53.48	387.54	56.92	151.79

App. XIA

Effect of laser, gamma and EMS treatments on peroxidase activity in inner true leaves and outer fleshy scale leaves on second and fourth day of sprouting in *A. cepa*

	Inner true leaves		Outer fleshy scale leaves	
Treatment	Day			
	2	4	2	4
Laser	Activity			
Control	20	8.3	0.27	0
476 nm				
200 mW -10′	9.8	29.41	0.203	0.23
400 mW -30′	21.73	25	0	0.19
488 nm				
200 mW -10′	14.28	15.62	0.23	0.26
400 mW -30′	9.8	18.51	0.27	0.277
Control	16.67	76.9	0.28	1.85
514 nm				
200 mW -10′	10.87	17.85	0	1.08
400 mW -30′	15.63	15.62	0.925	0.83
Gamma				
2 kR	25	1.25	1.4	0.28
5 kR	33.3	15.62	0.657	0
10 kR	27.7	33.33	5.6	0
15 kR	20	35.76	0.83	0
20 kR	20	20.83	1.01	0
EMS				
Control	-	12.82	-	-
0.2%	-	14.705	-	-
1%	-	20	-	2.747

App. XIIA

Effect of laser, gamma and EMS treatments on catalase activity in inner true leaves and outer fleshy scale leaves on second and fourth day of sprouting in *A. cepa*

Treatment	Inner true leaves		Outer fleshy scale leaves	
	Day			
	2	4	2	4
Laser	Activity			
Control	0.179	0.18	0	0
476 nm				
200 mW -10′	0.267	0.276	0	0
400 mW -30′	0.295	0.296	0	0
488 nm				
200 mW -10′	0.174	0.19	0	0
400 mW -30′	0.165	0.184	0	0
514 nm				
200 mW -10′	0.187	0.144	0	0
400 mW -30′	0.194	0	0	0
Gamma				
Control	0.165	0.184	0	0
2 kR	0.155	0.304	0	0
5 kR	0.173	0	0	0
10 kR	0.1732	0.31	0	0
15 kR	0.216	0	0	0
20 kR	0.155	0.23	0	0
EMS				
Control	0.301	0.198	0	0
0.2%	0.39	0.241	0	0
1%	0.388	0.198	0	0

App. IV B

Effect of UV- radiation at 1,2 and 3 hr on mitosis in *V. faba*

	Treatment			
Abnormalities (% of total cells)	Control	UV 1hr	UV 2hr	UV 3hr
Clumping	0.03	0.09	0.077	0.06
Stickiness	0.00	0.164	0.083	0.22
Nondisjunction	0.03	0.261	0.048	0.245
Bridges	0.01	0.024	0.042	0.022
Laggards	0.00	0.012	0.0179	0.0109
Micronucleate	0.02	0.00	0.048	0.00
Binucleate	0.02	0.23	0.17	0.20
Elongated nucleate	0.00	0.40	0.23	0.62
Total aberrations (%)	0.11	1.181	0.716	1.38
Total No. of cells observed	10792	16455	16758	18331
Mitotic index	3.74	2.91	3.12	2.72

App. V B

Effect of γ- irradiation at 2,5,10,15 and 20 kR on mitosis in *V. faba*

	Treatment					
Abnormalities (% of total cells)	Control	2 kR	5 kR	10 kR	15 kR	20 kR
Clumping	0.03	0.036	0.07	0.09	0.72	0.10
Stickiness	0.00	0.22	0.696	0.70	0.81	0.71
Nondisjunction	0.03	0.077	0.072	0.17	0.21	0.117
Bridges	0.01	0.057	0.096	0.15	0.18	0.196
Laggards	0.00	0.155	0.0338	0.0545	0.072	0.102
Micronucleate	0.02	0.48	1.50	7.91	9.48	9.58
Binucleate	0.02	0.34	0.23	0.19	0.12	0.117
Elongated nucleate	0.00	0.32	0.68	0.363	0.20	0.68
Total aberrations (%)	0.11	1.545 1.545	3.38 3.38	9.63 9.63	11.142 11.42	11.602 11.602
Total No. of cells observed	10792	19264	20666	16495	16701	12761
Mitotic index	3.74	3.09	2.74	2.37	1.79	1.54

App. VI B

Effect of EMS at 0.2, 0.4, 0.6, 0.8 and 1% concentration (30 min) on mitosis in *V. faba*

	Treatment					
Abnormalities (% of total cells)	Control	0.2%	0.4%	0.6%	0.8%	1%
Clumping	0.03	0.75	0.08	0.10	0.12	0.095
Stickiness	0.00	0.144	0.018	0.13	0.14	0.16
Nondisjunction	0.03	0.212	0.331	0.26	0.73	0.61
Bridges	0.01	0.06	0.078	0.08	0.05	0.089
Laggards	0.00	0.020	0.026	0.0293	0.0147	0.025
Micronucleate	0.02	0.14	0.22	0.35	0.073	0.14
Binucleate	0.02	0.164	0.25	0.329	0.33	0.21
Elongated nucleate	0.00	0.52	0.46	0.61	0.65	0.61
Total aberrations (%)	0.11	1.33	1.543	1.89	2.108	1.94
Total No. of cells observed	10792	14604	15310	13662	13589	15653
Mitotic index	3.74	2.7	2.49	2.12	1.72	1.37

App. VII B

Effect of HA at 0.2, 0.4, 0.6, 0.8 and 1% concentrations (1 hr) on mitosis in *V. faba*

	Treatment					
Abnormalities (% of total cells)	Control	0.2%	0.4%	0.6%	0.8%	1%
Clumping	0.03	0.087	0.095	0.10	0.126	0.114
Stickiness	0.00	0.07	0.154	0.131	0.210	0.186
Nondisjunction	0.03	0.31	0.178	0.403	0.338	0.529
Bridges	0.01	0.079	0.065	0.065	0.07	0.07
Laggards	0.00	0.0317	0.024	0.0216	0.03	0.0215
Micronucleate	0.02	0.032	0.012	0.0072	0.039	0.029
Binucleate	0.02	0.18	0.178	0.11	0.212	0.107
Elongated nucleate	0.00	0.39	0.210	0.65	0.43	0.62
Total aberrations (%)	0.11	1.18	0.907	1.492	1.455	1.67
Total No. of cells observed	10792	12612	16836	13882	12720	13973
Mitotic index	3.74	2.45	2.17	2.05	1.67	1.24

App. VIIIB

Effect of mutagens on meiosis and pollen sterility in *V. faba*

Sl. No.	Treatment	Total no. of pollen mother cells observed (PMCs)	Clumping of chromosomes (% of total PMCs)	Stickiness of chromosomes (% of total PMCs)	Bridges (% of total PMCs)	Laggards (% of total PMCs)	Unequal distribution of chromosomes (% of total PMCs)	Micronucleate (% of total PMCs)	Total aberrations (% of total PMCs)	Pollen sterility (%)
1.	Control	332	0.60	0.00	0.00	0.00	0.00	0.00	0.60	1.54
2.	476 nm 200mW-10′	229	2.62	0.87	0.437	0.00	0.44	0.00	4.37	4.77
3.	476 nm 400mW-30′	238	4.62	1.68	0.84	0.42	0.00	0.00	7.14	3.954
4.	488 nm 200mW-10′	450	8.89	0.88	0.44	1.33	0.44	0.66	12.64	2.89
5.	488 nm 400mW-30′	270	7.78	1.48	1.48	1.85	1.11	0.00	13.7	15.49
6.	514 nm 200mW-10′	145	3.45	2.76	2.069	0.00	2.069	0.00	10.35	8.11
7.	514 nm 400mW-30′	95	5.26	6.31	1.05	1.05	1.05	0.00	14.72	8.415
8.	UV 1hr	93	6.45	2.15	0.00	0.00	2.15	1.07	11.82	1.81
9.	UV 3 hr	252	6.75	1.59	0.00	0.00	1.98	0.00	10.32	5.84
10.	2kR	597	5.02	0.50	0.17	0.17	0.67	0.00	6.54	1.77
11.	5kR	117	11.11	5.12	1.71	0.85	0.85	0.00	19.64	3.60
12.	10kR	367	12.81	5.18	1.63	0.82	0.27	0.00	20.71	5.14
13.	15kR	96	15.6	6.25	2.08	2.08	2.08	3.125	31.215	3.94
14.	20kR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	32.78
15.	EMS (0.2%)	188	7.98	5.85	1.59	0.53	5.32	0.00	21.27	3.84
16.	EMS (1%)	18	11.11	5.55	11.11	0.00	11.11	0.00	38.88	54.58
17.	HA (0.2%)	232	5.17	4.3	0.86	0.00	2.59	0.00	12.92	2.30
18.	HA (1%)	275	5.45	1.45	1.09	0.36	1.82	1.45	11.62	18.47

App. IX B

Effect of mutagens on germination index, growth and yield in *V.faba*

Sl. No.	Treatment	Germination index	Plant height (cm)				Yield
			Days				Seed weight (g)
			20	40	60	80	
1.	Control	84	3.71	13.52	25.1		
2.	476 nm 200mW -10′	68	4.53	16.24	27.57		
3.	476 nm 400mW -30′	72	5.11	15.17	24.54		
4.	Control	96		11.33	22.25	28.68	5.05
5.	488 nm 200mW-10′	92		10.42	23.14	31.78	5.115
6.	488 nm 400mW-30′	96		12.67	29.39	38.29	6.74
7.	514nm 400mW-10′	92		11.52	25.55	35.4	5.33
8.	514nm 400mW-30′	88		9.77	25.32	34.19	4.73
9.	UV 1hr	92		10.09	24.72	34.45	5.31
10.	UV 3hr	88		10.23	21.04	29.09	4.06
11.	2kR	96		10.87	21.04	29.09	4.27
12.	5kR	96		8.62	17.96	28.56	2.96
13.	10kR	72		5.22	13.89	23.74	2.76
14.	15kR	8		9	31	36	10.22
15.	20kR	4		7	14	27	2.07
16.	EMS (0.2%)	100		9.6	23.96	32.95	3.53
17.	EMS (1%)	96		7.67	18.17	28.62	1.009
18.	HA (0.2%)	36		6.28	11.62	19.83	1.95
19.	HA (1%)	4		14.5	0.00	0.00	0.00

App. XB

Effect of laser, gamma and EMS treatments on soluble protein content in embryo and cotyledon on third and sixth day of germination in *V. faba*

Treatment	Embryo		Cotyledon	
	Day			
	3	6	3	6
	Soluble protein (µg/0.2ml)	Soluble protein (µg/0.2ml)	Soluble protein (µg/0.2ml)	Soluble protein (µg/0.2ml)
Laser				
Control	2538.6	1829.92	3021.4	2491.4
476 nm				
200 mW -10′	1925.3	2009.4	3099	3008
400 mW -30′	1976.1	1961.7	3485.5	3757.2
488 nm				
200 mW -10′	1427.4	1819	3408	3798
400 mW -30′	1672	2253	2976	2930
514 nm				
200 mW -10′	2237	1943	1163	1125
400 mW -30′	1989	1931	1137	1178
Gamma				
2 kR	1516.4	1840	3279.6	2760
5 kR	1458	1960	2269	3180
10 kR	1780	1600	3233	5040
15 kR	1422	2080	2757	3000
20 kR	1969	1680	4502	3010
EMS				
Control	1805.88	1539.58	4262.2	2138.6
0.2%	1100.8	1054.34	4430.6	2402.2
1%	2949.8	1078.3	3472.9	2600

App. XI B

Effect of laser, gamma and EMS treatments on protease activity and specific activity in embryo and cotyledon on third and sixth day of germination in *V. faba*.

Treatment	Embryo				Cotyledon			
	Day							
	3		6		3		6	
	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity
Laser								
Control	2.08	0.82	2.88	1.55	1.3	0.43	1.52	0.61
476 nm								
200 mW -10′	2.8	1.5	3.5	1.75	1.57	0.52	1.7	0.57
400 mW -30′	2.32	1.2	3	1.5	1.9	0.55	2.24	0.59
488 nm								
200 mW -10′	2.18	1.53	3.05	1.7	1.37	0.4	1.86	0.5
400 mW -30′	2.63	1.57	3.32	1.47	1.5	0.51	1.7	0.58
514 nm								
200 mW -10′	1.36	0.61	2.68	1.38	0.36	0.31	1.38	1.23
400 mW -30′	0.89	0.45	2.24	1.16	0.83	0.73	1.15	0.98
Gamma								
2 kR	2.68	2.32	2.6	1.41	2.15	0.66	2.07	0.75
5 kR	2.09	1.44	3.67	1.87	1.51	0.67	1.9	0.6
10 kR	1.87	1.05	3.83	2.4	1.63	0.5	1.822	0.36
15 kR	2.26	1.59	3.13	1.5	1.93	0.698	1.8	0.6
20 kR	2.34	1.19	3.43	2.04	2.38	0.53	1.63	0.54
EMS								
Control	2.18	1.207	2.486	1.6153	1.56	0.37	1.3	0.608
0.2%	1.51	1.373	1.503	1.426	1.21	0.273	1.38	0.574
1%	1.53	0.52	1.273	1.81	1.09	0.314	1.283	0.493

App. XII B

Effect of laser, gamma and EMS treatments on amylase activity and specific activity in embryo and cotyledon on second and fourth day of germination in *V. faba*.

Treatment	Embryo				Cotyledon			
	Day							
	2		4		2		4	
	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity	Activity	Sp. Activity
Laser								
Control	72.7	153.43	62.6	04.2	58.2	53.9	46.9	43.3
476 nm								
200 mW -10′	63.02	84.84	77.55	100.95	50.47	51.72	72.58	52.94
400 mW -30′	52.17	96.9	62.31	103.3	50.42	40.26	54.17	47.6
488 nm								
200 mW -10′	59.8	104.5	72.94	133.8	53.35	48.37	71.17	72.13
400 mW -30′	68.3	106.4	75.06	101.9	61.56	49.4	63.02	46.86
514 nm								
200 mW -10′	75.77	117	71.13	103.5	53.24	53.87	52.51	53.8
400 mW -30′	82.59	120	50.65	117.7	49.83	90.16	33.15	50.19
Gamma								
2 kR	83.95	212.8	89.78	154	90.76	101.46	93.33	141
5 kR	88.5	147.4	67.2	118.7	72.6	56.3	68.05	92.7
10 kR	80.1	169.8	68.05	128.30	60.62	50.85	60.9	68.16
15 kR	77.15	132.9	63.2	137	70	60.63	68.05	76.7
20 kR	61.27	119.1	64.05	297.9	64.2	60.7	75.2	99.3
EMS								
Control	50.252	89.73	78.67	163.25	47.31	33.41	62.31	45.53
0.2%	57.01	114.73	62.02	110.35	42.02	28.49	45.61	43.44
1%	44.08	47.95	64.84	114.96	50.55	37.95	45.61	43.44

App. XIV B

Effect of laser, gamma and EMS treatments on catalase activity in embryo and cotyledon on fifth day of germination in *V. faba*

	Embryo	Cotyledon
	Day	
	5	5
	Activity	Activity
Laser		
Control		1.44
476 nm		
200 mW -10′	-	1.01
400 mW -30′	-	0.7
488 nm		
200 mW -10′	-	1.014
400 mW -30′	-	1.3
514 nm		
200 mW -10′	-	0.56
400 mW -30′	-	0.32
Gamma		
2 kR	-	0.89
5 kR	-	0.96
10 kR	-	1.1
15 kR	-	1.14
20 kR	-	1.01
EMS		
Control		0.511
0.2%	-	0.358
1%	0.301	0.744