

Plant Genetics A View on the Effect of Laser Irradiation on Cell Division

Review Article

Norah M Al Aboud*

Department of Biology, Umm Alqura University, Makkah, Saudi Arabia

***Corresponding author:** Norah M. Al Aboud Department of Biology, Umm Alqura University, Makkah, Saudi Arabia
E-mail : nmaboud@uqu.edu.sa

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Abstract

The effect of lasers of wave length in the visible region 660-680 nm on mitotic cell divisions, germination and growth were studied in *Vicia faba*. The study indicated that lasers could be mutagenic causing aberration in the mitotic cell divisions while also producing changes in germination and growth of the plant. A wide range of chromosomal aberration were observed in all the four stages of mitotic cell division. The most important stickiness and non disjunction of changes observed were clumping chromosomes, bridges, laggards, micro nucleate, binucleate and elongated nucleate cells. In addition to these some other types of aberration such as inter phase with unequal sized nuclei, polyploid cells, chromatin droplets, nuclear bridges, nuclear polymorphism and multi nucleate conditions were also encountered in low frequencies. Laser exposure at 660-680 nm for 5 min caused increase in germination index in *V. faba* while germination index induced by exposures at 660-680 nm for 10 min was similar with control group. 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. Growth rate induced by 5 min of laser exposure was the highest one. The study suggests that laser may be used as a bio stimulator in agriculture. Further studies are required for elucidating the exact mechanisms by which lasers cause mutations.

Keywords: Laser irradiation; *Vicia faba*; Mutagenesis breeding; chromosomal aberration

Introduction

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 extra nuclear 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 such as they are generally recessive, but dominant mutations also occur, a small portion (0.1%) are beneficial but a majority of mutations are generally harmful to the organism, mutations may occur at random in any gene. However, some genes show higher mutation rates than the others and the same mutation may occur again and again, that is, they are recurrent.

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. Sub lethals kill more than 50% of the individuals while sub vitals kill much less than 50%. This type of mutations is 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 utilized in crop improvement.

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), these include various kinds of radiations such as Ionising radiations and Non ionizing radiations. Also we can induce mutations by certain chemicals mutagens (chemical mutagens) such as ethidium bromide and nitrous acid.

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 [1].

According to Evans [2] 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 mis repair of the lesions. Revell [3] have stated that all aberration are a consequence of exchange following a process of mis repair 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 utilized 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 [4]. 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 [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 [6]. Radiation between 510 and 610nm (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 carotenoids 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 bio molecules in a non-linear way.

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 photo ionisation or photo dissociation 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 assess the efficiency and effectiveness of laser ray mutagens in faba bean.
2. To study the effect of laser radiation in inducing chromosomal aberration during mitotic cell division by analysing the root tip squashes of *Vicia faba*.
3. To study the effect of laser radiation on mitotic index.
4. To study the effect of laser radiation on Germination and growth.

Review of Literature

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 [7].

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.

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 [8]. According to Blixt [9] the sensitivity of an organism depends up on the mutagen employed and its genetic makeup. 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 [10, 11]. 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 [10,11,13]. The difference in mode of action of mutagen [14] differential penetration of the mutagen to the target [15] efficiency of repair process [16]. and factors affecting the expression of concerned mutation [17] might also be playing a role in inducing mutations with varying frequency and spectrum. [18] and [19] 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 [20,21,17,22,23].

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 [24-26]. Reduction of these parameters has been attributed to various factors including changes in the balance of growth regulators and metabolic activity [27] physiological changes including inhibition of DNA synthesis [28] or inhibition of mitotic proliferation [29] Increase in seedling injury could be due to mitotic irregularities [30]. Sterility in pollen is mainly due to interchanges between nonhomologous chromosomes and detectable chromosomal aberration [31].

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 [2,32-35]. The suitability 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[33]. Several types of studies

have been done in this field so far for instance, [36] have studied the molecular mechanism in the production of chromosomal aberration with the 5 - Bromo deoxyuridine labelling method in *V. faba* while [37] studied the localisation of chemically induced chromosomal aberration in three different karyo types of *V. faba*.

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 (chromophore) to acceptor (DNA) produces breakage of phosphodiester bonds [38] 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 (lambda - 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/cm². The spectrum was wider at doses 1.28 and 1.72 J/cm². 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/cm². 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/cm². cv. *Auralia* proved more sensitive to this treatment and manifested higher mutability. Electro phoretic analysis of per oxidase showed that cv. *Auralia* reacted faster to the applied irradiation (lambda 488 nm).

[40] conducted a study to determine the laser irradiation effect on *Gossypium* seeds. Pre sowing 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.

Effect of lasers on germination and growth

Influence of laser beam of three different wavelengths - 337.1nm, 510 nm and 632.8 nm on germinating maize seeds was carried out to study some metabolic process in seedlings[41]. 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 24th 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.

[42] 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.

In 1986-1988 the effect of seed irradiation by laser on the vegetative and reproductive manifestations of the plants of the small fruit cultivar and pobeda cucumbers was investigated. Irradiation was performed with helium - neon laser of 632 nm wavelength and 20 mw power at the output, and with variants of one, three, five, seven and nine times. The strongest stimulation effect was obtained with five and seven - fold irradiation .with these variants the plants formed over ground vegetative mass by 10.1 and 15% 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 control by 16%), was obtained at seven - fold irradiation. The seed irradiation increased the content of dry mater, total sugars and vitamin C in the fruits and plastid pigments in their skin [43].

The effect of nitrogen laser (337.1 nm) and argon ion laser (514.5nm) irradiation on physiological response in the green gram *Vigna radiata* L. seedlings was studied by [44]. The shoot and root lengths and fresh dry weights of the seedlings increased with 30 min exposure to nitrogen laser and 5 min exposure to argon ion laser. Protein content was maximum with 20 min exposure to N laser and 5 min exposure to argon iron laser, while RNA and DNA contents were maximum at 5 min exposure with oth the laser treatments. [45] reported the results of pot and field experiments related to studies of the influence of laser irradiation on winter wheat, spring earley 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 interaction with that the described effects of laser irradiation occur in deteriorated environmental conditions.

Experiments were conducted in 1987-1988 by [46] 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 up to 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.

Materials and Methods

Materials

Taxonomically diverse plant species suitable for cytological

analysis, namely *Vicia faba* (faba bean) belonging to the family Leguminosae, sub family Fabaceae (family Leguminosae), was selected as the experimental material. Seeds of *V. faba* were obtained from local market.

Irradiation of samples by physical mutagens

The treatment included Laser irradiation at 660-680 nm (viz. 5 min exposures at power density 5 mW and 10 min exposure at the same power density). Laser source (5mW) was used for the irradiation *V. faba* seeds were soaked over night in tap water and decoated before irradiation. Seeds were divide into three groups after soaking, the first group was exposed to 5mW of laser radiation for 5 minute, the second group was also exposed to the same power for 10 minute and the therd group was the control group. Immediately after the various treatments, the faba bean germinated on moist filter paper in petridishes at 25⁺ 2°C. Faba bean root tips of the same size were collected between 10 and 11 A.M after 3-5 days of treatments.

Fixation of root tips

The root tips 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 up to 15 days in the refrigerator.

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°C 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 cover slip. The pressure was applied under several thickness of blotting paper.

Scoring of slides

For scoring of cytological aberration, temporary slides were used. Atleast 3 slides were prepared from actively dividing root tips in each dose and 9 fields (approximately 50-70 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. The percentage of prophase, metaphase, anaphase and telophase were calculated, also Mitotic index (M.I.) and total aberration were calculated as below:

Mitotic index M.I. % = $\frac{\text{Number of dividing cells}}{\text{Total No. of cells}} \times 100$

Total abnormality % = $\frac{\text{Total No. of abnormal cells}}{\text{Total No. of dividing cells}} \times 100$

Germination/Sprouting index studies

Germination/sprouting index was monitored in treatment of the mutagen used in this study, the germination/sprouting index was computed using the equation:

Germination index = $\frac{\text{No. of seedsshowing seedling(sprouting)}}{\text{Total No. of seeds}}$

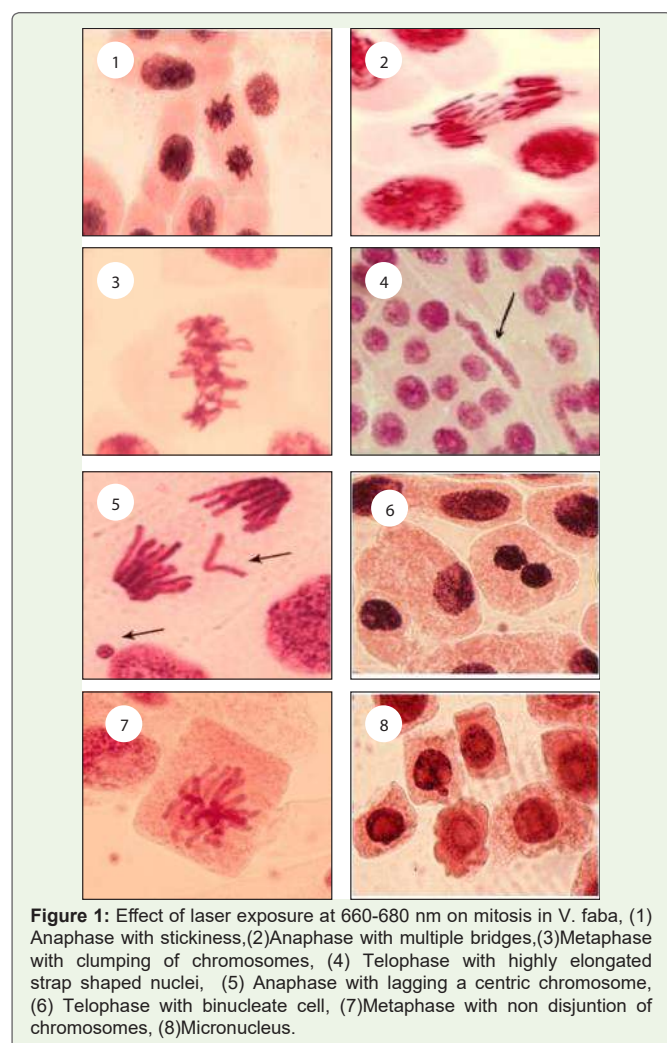
Results

Mitotic chromosome complement of the materials

The mitotic chromosome complement of *V. faba* contains 12 chromosomes which could be classified into five pairs of nearly equally long chromosomes with sub terminal centromeres (chromosomes II - VI, also referred to as the S chromosomes) and one pair with median centromere (chromosomes I, also called the M chromosome).

Mitotic chromosomal aberration

In control (untreated) samples mitosis was normal with only 0.17% of aberration observed. In treated samples, a wide range of chromosomal aberration were observed in all the four stages of mitotic cell division. The most important stickiness and non disjunction of changes observed were clumping chromosomes, bridges, laggards, micro nucleate, binucleate and elongated nucleate cells (Figure 1). In addition to these some other types of aberration such as inter phase with unequal sized nuclei, polyploid cells, chromatin droplets, nuclear bridges, nuclear polymorphism and multi nucleate conditions were also encountered in low frequencies.



Prophase aberration

Clumping of chromosomes: The most important type of chromosomal aberration observed at prophase was clumping of chromosomes (Table 1). The frequency of this change was very low (0.03%) in control (untreated) samples. Laser exposures at 660-680 nm (5 mW) induced an increase in clumping of chromosomes with increase in exposure time (Figure 2).

Metaphase aberration

Stickiness of chromosomes: The most common type of chromosomal aberration observed at metaphase was stickiness of chromosomes (Table 1). It was totally absent in the control but was encountered in all the mutagen treatments. Laser exposure at 660-680 nm (5 mW) induced an increase in stickiness of chromosomes with increase in exposure time (Figure 3).

Nondisjunction of chromosomes: Another common type of aberration observed at metaphase was non disjunction of chromosomes (Table 1). The frequency of this change was very low in control (untreated) samples (0.09%). Laser exposures at 660-680

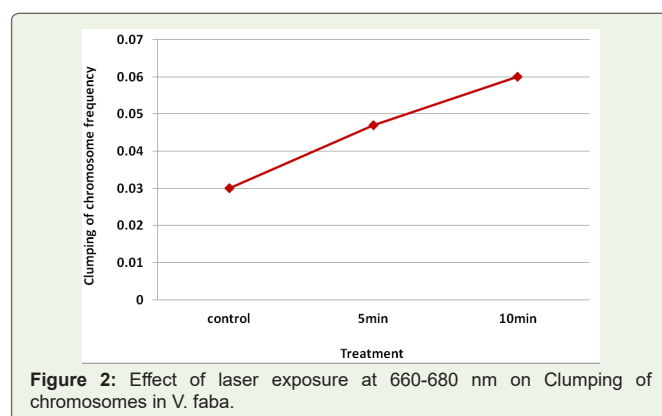


Table 1: Effect of laser exposure at 660-680 nm on seeds germination, plant height, mitotic cell division and chromosomal aberrations in *V. faba*

	Treatments		
	Control	5 min	10 min
Seeds germination%	60%	100%	60%
Plant height after 20 days	18.5	24.5	18
Plant height after 40 days	25	31	27
Clumping	0.03	0.047	0.06
Stickiness	0	0.085	0.13
Non disjunction	0.09	0.12	0.195
Bridges	0	0.194	0.145
Laggards	0	0.054	0.014
Micronucleate	0.06	0	0
Binucleate	0.05	0	0
Elongated nucleate	0	0.99	0.64
Total aberrations	0.17	1.49	1.097
Total No. of divided cells	307	422	342
Total No. of observed cells	6472	12867	11677
Mitotic index	4.74	3.28	2.93

Anaphase aberration

Bridges: The common type of aberration observed at anaphase were chromosome bridges (Table 1). Bridges were not found in control samples, while no time dependence could be established for the change in bridge frequency caused by laser exposures at 660-680 nm (Figure 5).

Laggards: Another type of aberration found at anaphase in low frequencies were chromosomal laggards (Table 1). This was not found in controls, and no time dependence could be established for the change in bridge frequency caused by laser exposures at 660-680 nm (Figure 6).



Figure 3: Effect of laser exposure at 660-680 nm on Stickiness of chromosomes in *V. faba*.



Figure 4: Effect of laser exposure at 660-680 nm on Nondisjunction of chromosomes in *V. faba*.

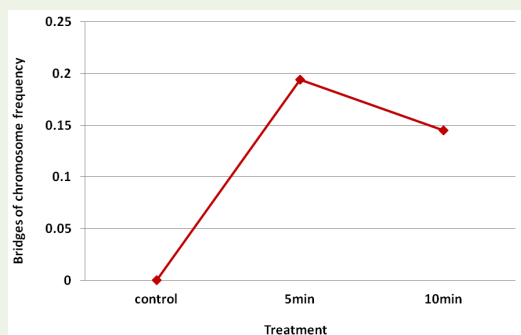


Figure 5: Effect of laser exposure at 660-680 nm on Bridges of chromosomes in *V. faba*.

Telophase aberration

Micronucleate cells: One of the aberration observed at telophase was cells with micronuclei (Figure7). It was seen in low frequency in the untreated controls.

Binucleate cells: Another type of aberration commonly found in most of the cases at telophase was the formation of binucleate cells (Figure8). It was seen in low frequency in the untreated controls.

Elongated nucleate cells: The presence of cells with highly elongated nuclei were another aberration seen at telophase (Table 1). It was absent in controls, laser exposures at 660-680 nm induced



Figure 6: Effect of laser exposure at 660-680 nm on Laggards of chromosomes in *V. faba*.



Figure 7: Effect of laser exposure at 660-680 nm on Micro nucleate of chromosomes in *V. faba* compared to the mutagen treated samples. laser exposures at 660-680 nm induced decrease in mitotic index with increase in time of exposure (Figure 11).



Figure 8: Effect of laser exposure at 660-680 nm on Binucleate of chromosomes in *V. faba*.

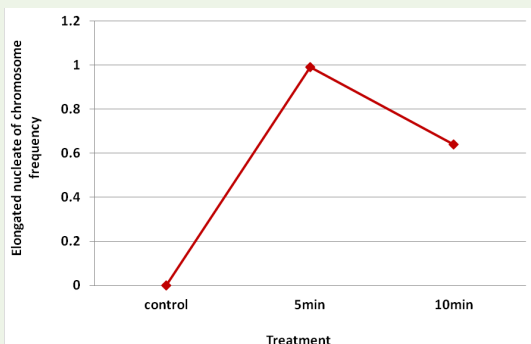


Figure 9: Effect of laser exposure at 660-680 nm on Total aberrations of chromosomes in *V. faba*.

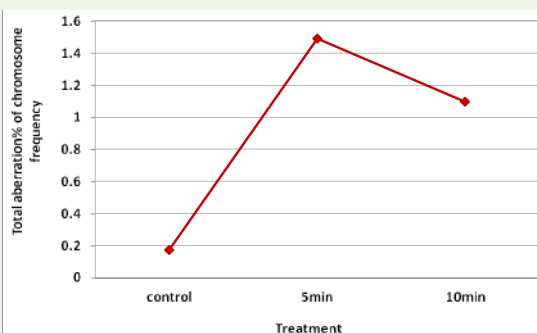


Figure 10: Effect of laser exposure at 660-680 nm on Total aberrations of chromosomes in *V. faba*.

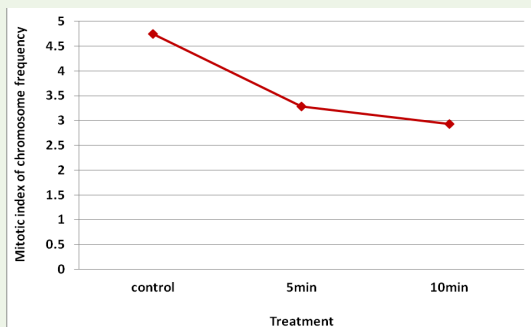


Figure 11: Effect of laser exposure at 660-680 nm on Mitotic index of chromosomes in *V. faba*.

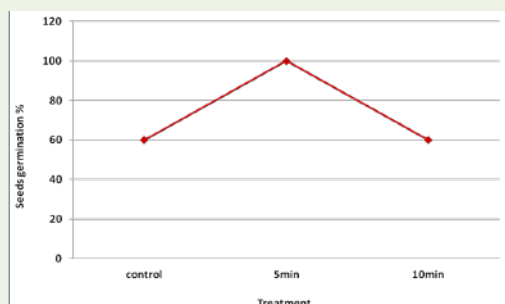


Figure 12: Effect of laser exposure at 660-680 nm on seeds germination % in *V. faba*.

increase in the frequency of the formation of elongated nuclei at the minimum exposure time (Figure 9).

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. Laser exposure at 660-680 nm induced increase in total aberration frequency at the minimum exposure time (Figure 10).

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 as



Figure 13: Effect of laser exposure at 660-680 nm on seeds germination in *V. faba*.

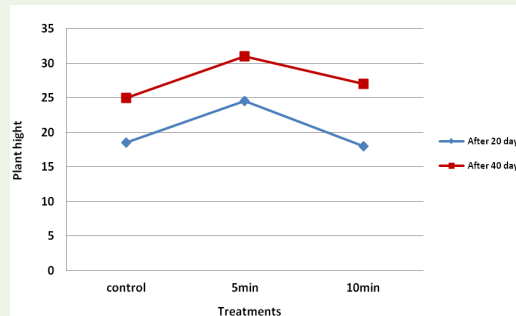


Figure 14: Effect of laser exposure at 660-680 nm on plant height after 20 and 40 days in *V. faba*.



Figure 15: Effect of laser exposure at 660-680 nm on plant height after 20 days in *V. faba*.

Germination/sprouting index

Laser exposure at 660-680 nm for 5 min caused increase in germination index in *V. faba*. While germination index induced by exposures at 660-680 nm for 10 min was similar with control group (Figure 12,13).

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 (Figure 14,15). Growth rate induced by 5 min of laser exposure was the highest one.

Discussion

Induced mutagenesis has been recently recognized as the most efficient tool for improving nutritional properties. Induced mutagenesis has played a significant role in successful development of new varieties by upgrading a specific trait without altering the original genetic makeup of the cultivar [47]. A single plant can contain a large number of different mutations resulting a manageable population sizes [48].

As per the reports of FAO, 2017 there are about 20 mutant varieties developed to date and the recently released mutant variety, Geca5, has improved attributes including good quality and early maturity.

Mutagenic effectiveness and efficiency have much importance in mutation breeding experiments. The measure of frequency of mutations induced by unit dose of mutagen is considered as mutagenic effectiveness; whereas the mutagenic efficiency represents the mutations on the basis of biological damage i.e. sterility, injury and lethality. In mutation breeding programmes, the mutagenic effectiveness and efficiency are necessary for obtaining desirable mutations in plants [49]. Mutagenic effectiveness and efficiency depends upon the type of genotype used and the mutagen applied on it.

Different workers have reported different effectiveness and efficiency of mutagens on different plants, e.g. grass pea [50, 51] lentil [52,53] clusterbean [54] chickpea [55] blackcumin [56] cowpea [57,58] garden pea [59] and mungbean [60]. Recently, laser irradiation has also been employed to meet agricultural demands and attain the goal of sustainable development [61,62].

Lasers of 5 mW (with wavelengths in the visible region 660-680 nm) caused aberration in the early 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 non disjunction of chromosomes. According to [63] the clumping of chromosomes may be attributed to the inhibition of protein synthesis.

Stickiness and non disjunction are due to disruption of bonds between protein and nucleic acid constituents or physical adhesion of protein aceous matrix resulting in failure of chromosome condensation in prophase [4,63,64]. According to [65] stickiness is the result of the breaks and chromosome exchanges during

prophase contraction. Apparently lasers affect the process of normal chromosome condensation.

Laser also apparently cause chromosome breaks, which are usually formed as a result of breaks in each arm of two adjacent chromosomes and their reunion was the most commonly observed type of stickiness. Multiple kinetochore chromosomes such as tracentric have been rarely observed, which apparently arise from breaks in more than two chromosomes. Polyploid cells were also observed rarely at metaphase. The spindle abnormality, polyploidy like 'regular orientation and scattered distribution of chromosomes at metaphase due to non synchronisation of division at centromere. Thus distribution of chromosomes is more confined to the periphery of the cell.

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. Bridges probably result from laser induced stickiness and bridge breakage fusion cycle [4]. 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. The frequency of Laggards was higher in samples that exposed to 5 min than those exposed to 10 min. One of the aberration observed at telophase was cells with micronuclei, it was seen in low frequency in the untreated controls.

Another common aberration seen in telophase is the formation of binucleate cells. It was seen in low frequency in the untreated controls. The formation of binucleate cells is attributed to the absence of cytokinesis [66] 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.

According to [67] changes in nucleic acid and protein synthesis changes cell volume or nucleus volume in mammalian cells.

In addition to these changes, The increased exposure time of treatments resulted in chromatin droplets in some cells and very rarely nuclear polymorphism and nuclear bridges were also encountered in some treatments. [66] suggested the term pseudonuclei for chromatin droplets resulted due to chromatin disintegration which remains scattered in the cytoplasm.

The total mitotic aberration induced by laser exposures was maximum at 5 min. Differences in the cytogenetic and mutational effects of different wavelengths of laser irradiation has been reported by several workers. For instance, [40] reported the dependence of mutation frequency *Gossypium* seeds on the irradiation intensity and [68] reported differences in mutation frequency in wheat dependent on time of irradiation with CO₂ lasers.

Previous study by Abdel-Fatah (2005) mentioned that moisture improved the effect of laser treatments on seed germination. The pre-sowing seed treatment with laser radiation stimulates the physiological and biochemical changes in the seed [69]. The treated

seeds with laser light can be applied to improve seed germination [70,71]. Similar results were observed on amaranth plant by [72] they mentioned that pre-sowing of seeds with laser light resulted in a significant increase of germination. Also, [73] reported that the germination of He-Ne laser irradiated seeds significantly increased as compared with control.

Many researchers reported that the stimulation effects of laser light are visible in sprouting seeds [74,75]. The laser light has a positive influence on enzyme activity and the concentration of free radicals in seeds [76,77,78] observed increasing in the activity of some phytohormones, especially indole-3-acetic acid (IAA) in the irradiated seeds.

Pre-sowing irradiation of seeds with laser light results in a faster uptake of water and achieving the larger mass during seed imbibing [79]. Also, amylolytic enzymes were increased in seeds after irradiation of the seeds with laser light improved germination [80]. Optimal doses of He-Ne laser irradiation may enhance FGP of *Negilla sativa*. Pre-sowing laser of seeds has imposed beneficial effects on germination, seedling growth and yield of various crops [62].

Laser irradiations had a stimulatory effect on growth and yield in faba. Growth rate induced by laser exposure of 5 min was the highest. Mutations affecting the plant height have been reported by several workers [81,82,83,84,85]. The plant height in mutants was reported to be affected by internodal length and alternations in number of nodes by Weber and [86] Gottschalk (1973) while [87] Bloustein and Gale (1984) proposed that the internodal length was probably affected by cell number, cell length or both.

Reduced growth in mutagen treatments due to auxin destruction, changes in ascorbic acid content and physiological and biochemical disturbances was noticed by several workers [88- 91]. Chromosome breakage during mitotic inhibition [92] and the effect of the mutagens on the metabolism of the organism [93] 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 [94] *Vigna radiata* seedlings [44] in winter wheat, spring barley and pea [45] in alfalfa and a grass mixture [46] false acacia forms [42].

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