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EFFECT OF CHEMICAL AND PHYSICAL MUTAGEN ON GENETIC VARIABILITY PARAMETERS OF FIELD PEA

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ABSTRACT

Ultraviolet radiation and acridine orange has been widely applied in agriculture for inducing genetic variability within plant species to be used in crop improvement, via induced oxidative stress in plant cell. Therefore, this study aimed to increase genetic variability in field pea (Pisum sativum L.) to choose the desired traits for crop improvement which depends on the presence of many genetic variations in the population. In this study one genotype of field pea was exposed to seven doses of physical and chemical mutagens to perform mutagenesis treatments. Over night pre-socked fresh seeds in tap water and or in 200 ppm acridine orange were exposed to different times of ultraviolet irradiation (UV) and then sown in the field. The dose of 45 minutes exposure to UV revealed the maximum mean value in root length if compared with the control. The most doses of mutagens observed significant decline in the number of nodules formed per plant, as well as induced significant increase in the concentration of chlorophyll b. High values of heritability coupled with high genetic advance as percentage of mean were obtained for plant dry weight, plant fresh weight, root length, number of nodules formed per plant, leaf area, chlorophyll a and chlorophyll b. This indicated the additive gene effect governed these traits and the selection will be effective for improving them in breeding programs. Whereas, low heritability estimates coupled with low genetic advance were obtained for the number of branches per plant, total chlorophyll, as well as carotenoids in leaves and pods, indicating that these traits were more affected by environmental factors. It is concluded that inducing genetic variations in field pea is useful in designing breeding programs either by selection or crosses.

Keywords: Acridine orange, ultraviolet rays, Pisum sativum L., nodulation, chlorophylls concentration, genetic parameters, heritability, genetic gain.

1. INTRODUCTION

Field pea is a legume crop having chromosome number 2n = 14 that offers both medicinal and dietary benefits. The medicinal importance due to decrease fats, sodium and cholesterols leading peas a valuable human dietary component, making it is used to prevent cardiovascular diseases (**Zilani *et al.* 2017**).

Pea (*Pisum sativum* L.) is one of crop legumes most cultivated worldwide, with a cultivated area increase than eight million hectare which produced over than 16 million tonnes per year, approximately 44% of which regarding to Europe **(Santos *et al.* 2019)**. It is a good model system in plant genetics since several scientific discoveries have obtained in pea by Gregor Mendel **(FAOSTAT 2014)**. Studying gene polymorphism in pea related to agronomically important parameters was essential to both principal and applied genetics on this legume crop **(Bohra *et al.* 2014)**. Modern pea genetics insights information on the genetic

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control of symbiotic interactions with nitrogen fixing bacteria **(Couzigou *et al.* 2012)**. A key event in the development of symbiosis between legumes and rhizobia is the activation of Rhizobia nodulation genes (*nod* genes). The final product of nodulation genes are the secreted lipo-chitin oligosaccharides, named as Nod factors, representing the material inducing initial nodule development in the host root system. Nod factors is a number of early symbiotic responses especially in root hair curling **(Spaink 2000)**. Rhizobial *nod* genes are activated by flavonoids present in root exudates. Flavonoids interact specifically with the end protein product of *nod* D gene. The active figure of *nod* D was activate transcription through *nod* operons promoters. This interaction was established in rhizobia that *nod* D is activated by *nod* D – flavonoid complex as *Rhizobium leguminosarum* biovar *viciae*, a microsymbiont with garden pea (*Pisum sativum* L.) **(Burn *et al.* 1987)**. *Rhizobium* formed symbiosis with *Pisum sativum* indicating several species within the genus *Rhizobium*. The strains originally isolated from pea root nodules were *Rhizobium leguminosarum* **(Frank 1889)**, *Rhizobium pisi* **(Ramírez-Bahena *et al.*2008)**, *Rhizobium indicum* **(Rahi *et al.* 2020)** and *Rhizobium ruizarguesonis* **(Jorrin *et al.* 2020)**. Out of these strains only *R. ruizarguesonis* was isolated from European countries like Italy and Germany **(Jorrin *et al.* 2020)**. Approximately 90% of the fixed nitrogen is transferred from bacteria to the plant **(Avcioglu *et al.* 2009)**.

Endophytic bacteria in different crop legumes could play a significant role to stimulate plant growth via exhibited nitrogen uptake, forming phytohormones (auxins and cytokinins), solubilization of minerals and iron chelation. They are probably suppress soil-borne pathogens via generating siderophores, antimicrobial metabolites or by competing the nutrients. Simultaneous infection with rhizobia exhibited nodulation and growth in wide variety of legumes. Legume crops have the potential role to remove soil contaminants via enhancing phyto-remediation **(Narula *et al.* 2013)**. Field pea (*Pisum sativum* L.) is a short duration crop legume grown in winter season containing high protein contents. It is used as a main vegetable crop in winter (green pods) in different parts of the world. It is one of the better food for human consumption, as well as for animal feeding and economical livestock **(Takeli and Ates 2003)**.

Elevated ultraviolet radiation reached to the Earth surface was released from the depletion of stratospheric ozone layer. The emission spectrum of the sun light containing 5-7 % UV light (200-400 nm). The largest spectrum of this radiation was absorbed in the atmosphere. Meanwhile, UV-C (100-280 nm) is completely absorbed, while UV-B (280-315 nm) was partially absorbed, as well as less than 0.5% of the total radiation reached the Earth surface classified in the spectrum of UV-B. This radiation even in low doses generated free radicals, as well as damaged the biological macromolecules. Therefore, living organisms should be able to interact specifically with UV-B to activate protection mechanisms. This radiation forming alteration in gene expression, as well as increased the contents of UV-absorbing compounds, and changes phytochemical content **(Lau *et al.* 2006)**. Therefore, the decrease in plant productivity are usually resulted from the combination of elevated ambient UV levels with another stress factors **(Singh *et al.* 2011)**. It has been stated that UV induces elevation in ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase, catalase and superoxide dismutase, and glutathione peroxidase **(Hideg *et al.* 2013)**.

For improving economical traits in diverse crops, mutation breeding was applied. In self-pollinated crops as *Pisum sativum* L. with limited genetic profile, mutation breeding is a potent beneficial methodology in the hands of plant breeders **(Micke 1988)**. The selection of effective mutagen is important for any mutation breeding programme to induce high frequency of desired

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mutation **(Singh and Singh 2001)**. Variability was essential for any breeding strategy to be induced genetic variations in the population as the basic requirement of developing crop varieties for sustainable crop productivity. Therefore, induced mutations was used to form beneficial variation in quantitative inherited traits **(Jagajananthan *et al.* 2012)**. Mutations generated new variations in the population leading to evolution of new species in nature to be using in breeding new varieties. Therefore, artificial mutations induced in crop varieties play a significant role in hybridization especially if the parents lost variability or deficient in desirable traits. Therefore, the genetic variability parameters as mean, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2), genetic advance (GA), as well as genetic advance as a percent of mean (GAM) were assessed to understand the extent of which the variations observed are due to the genetic factors or mainly affected by the environmental factors. Therefore, the present investigation was carried out to determine the nature of physical and chemical mutagen on the genetic variability parameters of physiological and growth traits in M_1 mutant population of field pea.

2. MATERIALS AND METHODS

This study was conducted in the experimental farm of Genetic Department located in the campus of Mansoura University during the winter season of 2022/2023.

Genetic materials

One genotype of field pea, *Pisum sativum* L., named Almomtaz, was used in this study. This genotype was kindly obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. This genotype was selected based on its availability, as well as economic importance in the vegetable market. The seeds were obtained from this source to avoid the heterogeneity of seeds.

Ultraviolet irradiation

The artificial source of ultraviolet rays was the UV lamp in the laminar cabinet located in Microbial Genetics Laboratory, Faculty of Agriculture, Mansoura University. The spectrum of this UV lamp belongs to high energy source named UV-B (280-320 nm) which is higher effective than UV-A for induced mutations **(Barta *et al.* 2004)**. The spectrum of UV lamp used in this investigation was 300 nm, leading to be classified as UV-B. Each minute of exposure time to UV lamp equal 188.2 joules/m^2 according to **Kondrateva *et al.* (2021)**. The joules were defined as one watt of irradiated power for one second.

Acridine orange

Acridine orange named N, N, N, N-tetramethyl acridine-3, 6-diamine is organic compound interact specifically with DNA by intercalation or RNA with electrostatic. It is able to penetrate the membranes of acidic organelles in the cell as lysosomes because it is capable to withstand in the low pH environments **(Narjes *et al.* 2019)**. Acridine orange intercalate between nitrogen bases in the DNA molecules caused distortion in DNA, as a consequence DNA polymerase recognized this stretch as additional base, formed frame shift mutations that varied the reading frame of DNA which changed the gene message from the starting point of addition or deletion producing rearrangement of code bases sequence in the DNA and thereby altered the

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amino acids sequence in the generated polypeptide chain to became a mutant protein **(Oladosu *et al.* 2016)**.

Acridine treatment

Total 600 seeds were immersed in tap water for six hours to initiate pre-soaking. Such pre-soaked seeds were later immersed in chemical mutagen solution for six hours with continuous shaking. Pre-soaking exhibited the rate of mutagen uptake by exhibited cell permeability, as well as initiates the metabolism in the seeds for mutagenic treatment. To ensure the absorption of mutagen, the size of mutagenic solution employed was six times than that of the seeds. The seeds immersed in tap water for 12 hours served as control. The acridine orange concentration used in this study is 200 ppm. The seeds were properly rinsed under running tap water after the mutagen treatment.

Physical mutagen treatment

To irradiate the seeds with ultraviolet rays, 600 seeds were employed. The seeds were immersed in tap water for 12 hours and then exposed to different times of UV rays (15, 30 and 45 minutes) in the laminar cabinet containing UV lamp.

Physical and chemical mutagenic treatment

The interaction between physical and chemical mutagen was studied via used 300 seeds immersed in tap water for six hours and then immersed in mutagenic solution for another six hours to be exposed with different doses of UV rays (15, 30 and 45 minutes) in the laminar chamber supported with UV lamp. Treatment details are furnished in **Table 1**.

Table 1. Treatment details.

Treatments	Mutagen (chemical/physical)	Concentration/Dose
T_1	Untreated control	0.0
T_2	Acridine orange (AO)	200 ppm
T_3	Ultraviolet rays (UV)	15 minutes
T_4	Ultraviolet rays (UV)	30 minutes
T_5	Ultraviolet rays (UV)	45 minutes
T_6	Acridine orange (AO) + Ultraviolet rays (UV)	200 ppm + 15 minutes
T_7	Acridine orange (AO) + Ultraviolet rays (UV)	200 ppm + 30 minutes
T_8	Acridine orange (AO) + Ultraviolet rays (UV)	200 ppm + 45 minutes

Experimental design

The field experiment was performed in a completely randomized block design with three replications. The seeds were sown in the field trial to obtain M_1 populations. The seeds were sown in ridges by adopting a spacing of 30 cm between the plants and 90 cm between the ridges. The plants were carefully thinned 15 days after emergence with two plants were maintained in each individual hill. The experiment was irrigated regularly with river water under the open field condition. All the intercultural activities including plant protection were carried out as per the recommended package of practices by Egyptian Ministry of Agriculture. Observations were recorded from individual plants in each treatment as; number of branches per plant at the harvest time, plant fresh weight at 50 day-plant old, plant dry weight at 50 day-plant old, number of

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nodules per plant at 50 day-plant old, root length (cm) at 50 day-plant old, leaf area (cm^2) at 50-day plant old, chlorophyll in leaves at 50 day plant old, as well as chlorophyll and carotenoids in pods after the pods were completely formed. In this investigation growth and yield traits were measured in M_1 individual plant that may carries dominant mutant phenotype. The significant of M_1 is ensuring maximum variability in their survival which is beneficial. Most mutations are recessive in nature and cannot be observed in heterozygous states. Meanwhile, dominant mutants were only observed in heterozygous state of M_1 generation, but recessive mutations were observed in homozygous state of M_2 generation, after the seeds were formed by fusion between male and female gametes that are carries the same mutation **(Kumar *et al.* 2019)**.

Dry matter accumulation and assessment photosynthetic pigments

Three plants from each sector were uprooted at 50 day plant old. The uprooted plants were carefully washed weighted and oven-dried. The dry matter was subsequently determined after oven dried at 70 C^o until reached to the constant weight. Chlorophyll content was measured according to the method of **Arnon (1949)**. Meanwhile, the carotenoid content was measured by the formula suggested by **Krik and Allen (1965)**.

Symbiotic attributes

Nodules detached at 50 day plant old from the root system of untreated (control) and mutagen-treated plants were separated and counted for each individual plant according to **Shahid *et al*. (2018)**.

Genetic parameters

Genetic variability parameters like genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), environmental coefficient of variation (ECV), heritability in broad sense (h^2), genetic advance (GA) and genetic advance as a percent of mean (GAM) for all traits studied in this investigation were determined to understand the extent to which the variance obtained due to genetic factors or referred to environmental effects. This assessments were worked out according to **Bammanakatti *et al.* (2023)**. Genotypic and phenotypic coefficient of variation were classified as low (0-10%), moderate (10-20%) and high (above 20%) according to **Sivasubramanian and Madhava Menon (1973)**. Heritability percentage was classified as low (0-30%), moderate (30-60%) and high (above 60%) according to **Robinson *et al.* (1949)**. Genetic advance as percentage of mean was classified as low (0-10%), moderate (10-20%), as well as high (above 20%) according to **Johnson *et al.* (1955)**.

Statistical analysis

Results are the mean values of three biological replicates from each sector. The data were subjected to analysis of variance (ANOVA) to test the significance of differences between treatment means using F-test. The least significance of differences (LSD) was used to compare between means according to **Gomez and Gomez (1984)**.

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3.RESULTS AND DISCUSSION

Growth parameters

To create a new genotypes in plant population, the use of chemical or irradiation mutagens is an interesting technique which had becomes an established biotechnology. In this method many induced mutants have been released as cultivars **(Maluszyonski *et al.* 1995)**. **Table 2** showed the data of plant growth parameters. It is obviously that the dose of 200 ppm acridine plus 30 minutes of exposure time to ultraviolet irradiation possessed significant mean values for plant fresh weight, plant dry weight and root length above the control. There were a significant increase in the mean values of root length than the control at the doses of 30 minutes, 45 minutes of ultraviolet irradiation (UV), 200 ppm acridine plus 15 minutes of UV and 200 ppm acridine plus 30 minutes of UV. The results revealed that the dose of 45 minutes of UV showed the maximum mean value of root length which gave extreme mean value (19.5) if compared with the control mean (10.4). The root length was ranging from 9.83 to 19.50 cm at 15 and 45 minutes of exposure time to UV, respectively. The M_1plants showed irregular variations in root length at different doses of mutagenic agents. Similar reports was observed before by **Gnanamurthy *et al.* (2012)** in cowpea and **Rukesh *et al.*(2017)** in green gram. The dose of 45 minutes of exposure time to UV registered the maximum total of root length increased. The effect of irradiation maybe due to seeds metabolism and onset of DNA synthesis **(Shah *et al.* 2008)**. These results indicated that the same dose of mutagens showed differences in their effects on the different economical traits. On the other hand, some doses stimulated cell division to be increased root length.

Table 2. Effect of acridine and ultraviolet irradiation on growth parameters of field pea.

Treatment		Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Number of branches/plant	Number of nodules/plant	Leaf area (cm^2)
00		10.7	1.57	10.40	8.0	55.3	473.00
200 mg AC		14.8	1.63	11.90	5.7	52.3	363.03
15 min UV		6.3	1.37	9.83	7.0	38.0	384.97
30 min UV		7.1	1.21	14.30	5.7	27.0	226.93
45 min UV		7.9	0.74	19.50	9.3	12.7	459.83
200 mg AC + 15 min UV		14.4	0.08	14.16	6.0	34.0	449.03
200 mg AC + 30 min UV		18.2	2.38	12.83	7.3	26.3	631.90
200 mg AC + 45 min UV		11.1	1.27	11.75	8.0	33.0	164.73
Grand mean		11.31	1.28	13.08	7.125	34.825	394.18
Treatment mean		11.4	1.24	13.47	7.0	39.80	382.92
F-test		**	**	**	Is	**	Is
LSD	0.05	2.79	0.43	2.15	3.24	4.25	285.34
	0.01	3.87	0.60	2.99	4.50	5.90	396.02

*, ** = Significance at 0.05 and 0.01 probability levels, respectively.
Is = Insignificant differences. LSD = Least significant difference
Ac = Acridine orange UV = Ultraviolet rays.

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The observed modifications of growth in response to mutagens may be attributed to changes in membrane permeability, transpiration and the opening of stomata **(Roy 1974)**. Mutagens can also generate changes in the activity of endogenous plant growth regulators and may in turn influence plant growth parameters **(Maherchandani 1975)**. Alteration in membrane permeability may decreased the absorption of some nutrients as calcium which played a significant role in various growth parameters and development processes **(Sanders *et al.* 2002)**. Reduction in calcium absorption leads to generates yellowed leaves, decrease plant growth, breakdown cell walls, as well as increased sensitivity of plant to pathogens **(Medvedev 2005)**. The results agreed with **Dhawi and Khayri (2009)**, who reported that magnetic field inflicted alterations in chlorophyll, DNA and water content as well growth expressed in plant fresh weight. These parameters were also modified by mutagens treatment. Exposure to mutagens suggested to inflict physiological stresses leading to inducing growth and physiological modifications **(Ahloowalia and Maluszynski 2001)**. Some reports provide evidence of stimulating effect on plant growth if the seeds were exposed to low-doses of ionizing radiation **(Zaka *et al.* 2004, Mortazavi *et al*, 2006)**. The sensitivity to radiation is dependent upon several factors as radiation type, radiation dose, physiological status and plant genotype **(Tabasum *et al.* 2011)**.

The results also showed significant differences between treatments in the number of nodules formed per plant which showed significant decline at most doses of treatments. Ultraviolet irradiation and the interaction between UV plus acridine are drastically reduced nodule formation. The reduction in nodule formation in relation to the control may be due to the effect of mutagens on the metabolite exchange between the host plants and bacteria including exchanges with signal molecules which played a direct role in the nodulation process of legume host **(Calatrava-Morales *et al.* 2018)**. These results agreed with **Ivanova *et al.* (2015)**, who found several mutant lines in field pea with impaired nodule formation which considered to be a reasonable approach for further characterization of gene networks that operate during nodule development. These results imply place about ultraviolet irradiation, as well as the interaction between UV plus acridine in reducing nodulation. The irradiated plants established lower effective symbiosis with pea plants. The inhibitory effect of mutagens on the number of branches and leaf area developed per plant was attributed to the effects of mutagens on the physiological system affects on cell division rates, as well as on activation of growth hormone **(Zaka *et al.* 2004)**. The role of plant genetic factors governing flavonoid production which may affected by mutagenic agents has been confirmed by **Firmin *et al.* (1986)**, who found a negligible *nod* gene - inducing activity in the exudate of roots in white flowering mutant of *Antirrhinum*. In addition, **Hungria and Phillips (1993)**found less release of inducers from bean seeds with light test a pigmentation. Meanwhile, **Kapulnik *et al* (1987)** obtained correlation between luteol synthesis from alfalfa roots and nodulation in breeding experiments. Constitutive expression of *nod* genes led to decreased nodule number, as well as failure in nitrogen fixation, while an increased dose of *nod* genes prevented nodulation **(Knight *et al.* 1986)**. It is conceivable that the inability of the deregulated strains to switch off *nod* gene activity at the stage of nodules development caused damage to nodules and their function **(Schlaman *et al.* 1991)**.

The decline obtained in symbiotic attributes such as nodule number developed per plant may be due to the deterioration of growth regulatory enzymes involved in the progression and improvements of legumes or may be attributed to the distraction of signaling between

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phytochemicals as luteolin, apigenin and *Nod* D receptors which are the fundamental components for the initiation of nodule developments and nitrogen fixation **(Fox *et al.* 2007)**.

Table 3. Effect of acridine and ultraviolet irradiation on chlorophyll and carotenoid concentration.

Treatment		Chlorophyll in leaves (mg/g FW)			Carotenoid (mg/g FW)	
		a	b	Total	Leaves	Pods
00		0.14	0.15	0.29	1.60	0.65
200 mg AC		0.03	0.40	0.44	1.27	0.59
15 min UV		0.06	0.14	0.20	1.22	0.52
30 min UV		0.15	0.38	0.53	1.34	0.49
45 min UV		0.01	0.11	0.12	1.40	0.51
200 mg AC + 15 min UV		0.02	0.35	0.37	1.16	0.42
200 mg AC + 30 min UV		0.04	0.39	0.43	1.57	0.47
200 mg AC + 45 min UV		0.04	0.41	0.45	1.41	0.36
Grand mean		0.06	0.29	0.35	1.37	0.50
Treatment mean		0.05	0.31	0.36	1.34	0.48
F-test		**	**	**	Is	*
LSD	0.05	0.03	0.15	0.14	0.30	0.18
	0.01	0.04	0.20	0.19	0.42	0.24

*, ** = Significance at 0.05 and 0.01 probability levels, respectively.
Is = Insignificant differences. LSD = Least significant difference
Ac = Acridine orange UV = Ultraviolet rays.

Photosynthetic pigments

As shown in **Table 3**, the photosynthetic pigments in leaves responsed to mutagenic agents were differed between chlorophyll a which showed decline in chlorophyll concentration and chlorophyll b which showed increase in their concentration above the control plants. The most doses of mutagenic agents showed significant increase in chlorophyll b as well as in total chlorophyll.

Carotenoid concentration in leaves showed insignificant differences between treatments in contrast with its concentration in pods which showed significant differences between treatments. The chlorophyll a : b ratio was more variable amongst the same treatment through exhibited increase in chlorophyll b concentration in relation to the concentration of chlorophyll a. In leaves and pods, the carotenoid concentration was decreased in response to all doses of mutagens in relation to the control. The decline obtained in chlorophyll a and carotenoid in leaves and pods may be resulted from immature leaves and pods had not yet developed protection mechanisms and direct photo oxidation of pigments could occurred. Immature leaves and pods do not have generated cuticle wax layers that are shown to act as protectors in preventing a decline in photosynthesis under mutagenic treatments **(Skórska 2000)**. These results agreed with **Casati**

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and walbot (2004), who found that immature leaves have specific gene expression pattern affecting on the developmental processes leading to be differed than adult leaves or pods in their transcriptome changes upon mutagens treatment. This is probably the reason of immature leaves being not be able to react efficiently with mutagens as mature leaves.

Elevated mutagen doses has led mainly to decline in photosynthetic pigments content due to reduced carbon allocation to chlorophyll synthesis leading to increased chlorophyll degradation **(Lau *et al.* 2006)**.The increase in total chlorophyll pigments might be due to the increase in chlorophyll b concentration at most doses of mutagens. This increase may be due to up-regulation of chlorophyll protection enzymes and carbon allocation reaching their maxima under the most doses of mutagenic agents. This protection mechanisms protect chlorophyll pigments from the up-regulation of chlorophyll degrading enzymes. In this study, the mutagenic agents had differed effect on chlorophyll a and chlorophyll b contents, thus leading to changing the chlorophyll a : b ratio as reported by **He *et al.*(2006).**The elevation of pigment concentration under the most doses of mutagenic agents could lead to increased photo synthesetic activity, as well as more efficient photoprotection, as decided during the first days of UV irradiated *Indigo feratinctoria* by **Ravindran *et al.*(2008).** Synthesis of phenolic compounds is the first line of defense when the plants were irradiated with ultraviolet rays **(Landry *et al.*1995)**.Formation of UV absorbing components decrease the dose of radiation that penetrates the cells, thus lowering damage to macromolecules **(González *et al.* 1996)**. It has been shown by **Bieza and Lois (2001)** that exposure to UV-B radiation exhibited the content of total phenolic compounds known as the first line of plant defense against the mutagens that penetrates the cell leading lowering effect on macromolecules **(González *et al.* 1996)**. Most doses of mutagens enhanced chlorophyll b and decline chlorophyll a concentration as compared to the control. It was observed that chlorophyll b was greater than chlorophyll a. These results agreed with **Hamideldin and eliwa (2015)**, who found that photosynthetic pigments in the leaf of mustard plants was increased in response to gamma irradiation. Increased chlorophylls by irradiation maybe due to biosynthesis stimulation or delaying chlorophyll degradation **(Aly *et al.* 2018)**. Furthermore, increased photosynthesis in irradiated plants may be participates to increase plant growth**(Wi *et al.* 2007)**.

Concerning carotenoids content, all doses of mutagens were markedly decreased carotenoid content in leaves and pods. The highest carotenoids content in leaves and pods was achieved from unirradiated plants without significant differences between treatments for leaves carotenoids. The lowest carotenoids content in pods was achieved from the plants irradiated with 200 ppm acridine plus 45 minutes of exposure time to ultraviolet rays. Increases in carotenoids content are significant way to exhibit plant resistance to abiotic stresses **(Shala and Mahmoud 2018)**. These results agreed with **Masoud *et al.*(2018)**, who found that carotenoids content in chicory plants increased with the increases of gamma doses as a defensive impact and the highest content of carotenoids was recorded from the plants irradiated with 80 Gy. Photoreceptor pigments (chlorophylls and carotenoids) play a significant role in the development of organic molecules as carbohydrates and proteins **(Guidi *et al.* 2017)**. Under abiotic stress condition, chlorophyll content and other photosynthetic pigments generally decreases as seen in this study for chlorophyll a in leaves, as well as for carotenoids contents in leaves and pods which declined at all doses of mutagens **(Sankar *et al.* 2017)**. Therefore, it seems probably that mutagens doses used in this study might have inhibited the activity of the enzymes related to photosynthetic carbon reduction cycle as 3 PGA kinase, NADP, aldolase and NAD-glyceraldehy-3-P dehydrogenase. The study of numerous photosynthetic pigments and fluorescence parameters of

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plants raised to abiotic stressed environments validated that the light reactions of photosynthesis are highly sensitive to the exposure of chemicals **(Junqueira *et al.* 2017)**.

Genetic parameters of growth traits

The availability of genetic diversity and knowledge of the genetic parameters about quantitative traits are prerequisites for crop improvement. In order to exhibit field pea productivity, the assessment of genetic variability among the genotypes induced by mutagenic doses and understanding the genetic parameters are the core activities of pea breeding. The estimates of genetic parameters for sex quantitative traits are presented in **Table 4**. Both genotypic and phenotypic coefficient of variation (GCV and PCV) were higher for plant dry weight, plant fresh weight, root length, number of branches per plant, number of nodules developed per plant and leaf area. The existence of variability among the genotypes induced by mutagenic agents reflected a significant contribution of genotypic variance to the observed variation. High phenotypic coefficient of variation coupled with high genotypic coefficient of variation ensure the existence of sufficient variation induced by the mutagenic agents within the study genotype in regard to these traits. These results indicated that the observed phenotypic variation was mainly attributed to genetic effect because GCV and PCV values were very close to each other for all quantitative traits studied in this investigation.

Table 4. Estimation of genetic parameters for some agro morphological traits.

Growth parameters	Variance components			Genetic variability		
	Genotypic variance (σ^2g)	Phenotypic variance (σ^2p)	Environmental variance (σ^2e)	GCV (%)	ECV (%)	PCV (%)
Plant dry weight	0.27	0.33	0.06	15.77	1.65	17.43
Plant fresh weight	16.73	19.28	2.54	42.99	3.16	46.15
Root length	8.75	10.26	1.51	28.91	2.39	31.30
Number of branches per plant	0.57	3.99	3.42	10.00	16.45	26.45
Number of nodules per plant	193.11	199.01	5.90	83.25	1.25	84.51
Leaf area	12825.48	39369.68	26544.20	201.67	151.65	353.33

Table 4. Continued.

Growth parameters	Genetic advance		
	Heritability (%) (H^2bs)	Expected genetic advance (EGA)	GAM (%)
Plant dry weight	90.45	1.37	100.84
Plant fresh weight	93.15	10.85	95.93

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Root length	92.35	7.86	60.09
Number of branches per plant	37.79	2.00	28.08
Number of nodules per plant	98.51	36.96	106.13
Leaf area	57.07	301.23	76.42

GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation, PCV = phenotypic coefficient of variation, GAM = Genetic advance as a percentage of mean.

High heritability values were recorded for the number of nodules developed per plant (98.51%) followed by plant fresh weight (93.15%), root length (92.35%), plant dry weight (90.45%) and leaf area (57.07%). High values of heritability validated the great importance of the genetic factors on the phenotypic of different quantitative traits. The highest genetic advance as a percentage of mean was recorded for the number of nodules developed per plant (106.13) followed by plant dry weight (100.84%), plant fresh weight (95.93%), leaf area (76.42%) and root length (60.09%). Greater contribution of genetic variance and availability of enriched variability suggested that phenotypic selection would bring a progressive genetic gain. The results indicated that these traits governed by additive gene effects as evidenced by high values of heritability coupled with high GAM. Heritability values provided details on the level of genetic influence over the manifestation of certain traits and the accuracy of phenotypic prediction of breeding value. Breeding techniques usually taken heritability to estimate of how well desirable genes were passed on from parents to offspring **(Falconer 1996)**. The number of branches developed per plant showed a great difference between GCV (10.0%) and PCV (26.45%), indicated the greater contribution of environmental effect on observed phenotype **(Bandila *et al.* 2011)**. This leading to obtain low value of heritability coupled with low genetic advance, which suggestes that selection of elite genotypes for higher branches developed per plant should undergone in the later generations until the fixation of desirable alleles contributing in developing branches. These results indicated a high contribution of environmental factors on the observed variability of branches number developed per plant. Hence, direct selection for the number of branches developed per plant would be ineffective for the improvement of this trait, indicating low scope of selection for improvement of this trait. Assessment the phenotypic coefficient of variation gives the extent of variability present in the population, meanwhile the genotypic coefficient of variation alone does not reflect the heritable variation present in the population.

Heritability and genetic advance are important selection parameters which help to understanding the mode of inheritance of different quantitative traits. Therefore, heritability values and genetic advance as per cent of mean would give information an idea about the nature of gene action governing a particular traits. The results obtained in this study are in harmony with results obtained by **Kalaiyarasi *et al.* (2019)**, who obtained high heritability in *Sesamum indicum* L. for the seed yield per plant, number of branches per plant, plant height, number of capsules per plant and seeds per capsule. But low genetic advance as percentage of mean observed by the same authors for days of fifty percent flowering. The traits showed high heritability estimates as plant fresh weight, plant dry weight, root length, number of nodules developed per plant and leaf area, indicates that selection for these traits would give the best results for improving these traits. This reflected that these traits were controlled by additive gene

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action and observe better scope for improvement through direct selection. This agrees with **Katore and Navale (2018)**, who obtained high heritability coupled with high genetic advance in *Pisum sativum* L concerning plant height, seed yield per plant, number of pods per plant and days to maturity. On the other hand, **Gudmewad *et al.* (2018)**obtained high heritability coupled with high genetic advance in linseed for days to 50 percent flowering, plant height, number of primary branches per plant, number of secondary branches per plant, number of capsule per plant and 1000-seed weight.

Genetic parameters of biochemical traits

As shown from the results tabularized in **Table 5**, the genotypic coefficient of variation (GCV) was slightly lower than phenotypic coefficient of variation (PCV) for chlorophyll a, chlorophyll b and carotenoid in leaves, indicating minor environmental effects on gene expression of these traits. High heritability values coupled with high genetic advance were observed for chlorophyll a and chlorophyll b.

Table 5. Estimation of genetic parameters for biochemical traits.

Biochemical traits	Variance components			Genetic variability		
	Genotypic variance (σ^2g)	Phenotypic variance (σ^2p)	Environmental variance (σ^2e)	GCV (%)	ECV (%)	PCV (%)
Chl (a)	0.002	0.0028	0.0003	7.22	0.419	7.639
Chl (b)	0.014	0.021	0.007	7.75	1.74	9.49
Total Chl	0.003	0.0063	0.006	1.029	3.70	4.72
Carotenoids in leaves	0.016	0.046	0.03	3.81	2.65	6.47
Carotenoids in pods	0.003	0.013	0.01	2.73	2.96	5.69

Table 5.Continued.

Biochemical traits	Genetic advance		
	Heritability (%) (H^2bs)	Expected genetic advance (EGA)	GAM (%)
Chl (a)	89.51	0.13	209.12
Chl (b)	66.66	0.26	89.27
Total Chl	21.77	0.04	12.57
Carotenoids in leaves	34.78	0.20	14.45
Carotenoids in pods	48.03	0.15	29.20

GCV = Genotypic coefficient of variation, ECV = Environmental coefficient of variation, PCV = phenotypic coefficient of variation, GAM = Genetic advance as a percentage of mean.

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The phenol typic coefficient of variation (PCV) was greater than genotypic coefficient of variation (GCV) for total chlorophyll and carotenoids in pods, indicating that the environmental factors had an important role in the gene expression of these traits. Low heritability values were coupled with low genetic advance as percent of mean for total chlorophyll and carotenoid in leaves. Meanwhile, moderate heritability estimates were coupled with moderate genetic advance as percent of mean for carotenoid in pods. Therefore, heritability estimates along with excepted genetic gain is more important than heritability estimates alone in predicting the resultant effect for selecting the superior genotypes **(Johnson *et al.* 1955)**. As reported by **Sivasubramanian and Menon (1973)** about the PCV and GCV estimates are ranked as low (0-10%), medium (10-20%) and high (>20%). **Sudhakar *et al.* (2007)** observed low phenotypic and genotypic coefficient of variation for days to fifty percent flowering, days to maturity and oil content in sesame. Hence, **Johnson *et al.*(1955)** categorized genetic advance as percent of mean to high (>20%), moderate (10-20%) and low (0-10%). Accordingly, high heritability values coupled with high genetic advanced as percent of mean were observed for chlorophyll a and chlorophyll b, indicated that these traits were controlled by additive gene effects, as well phenotypic selection for these traits would likely to be effective. This indicating lesser influence of environmental factors in the gene expression of these traits and prevalence of additive gene action in their inheritance. Thus, heritability is a good indicator for the transmission of traits from parents to their offspring **(Falconer 1989)**. The estimates of heritability are more advantageous if expressed in terms of genetic advance. It is not necessary that a trait observed high heritability values will also exhibit high genetic advanced **(Johnson *et al.* 1955)**.The assessment of heritability will help plant breeder in selection of elite genotypes from diverse genetic population. Genotypic coefficient of variation is not a correct pathway to know the heritable variation available in the population and should be considered together with heritability assessment **(Tigabu *et al.*2021)**. High values of heritability indicated that there were a close correspondence between the genotype and the phenotype which reflected relative small contribution of the environment on the phenotype. Hence, the traits with low heritability values, say40% or less, as total chlorophyll and carotenoid in leaves, making selection may be considerably difficult due to the masking effect of the environment. The results obtained in this study agreed with **Nithinkumar *et al.* (2022)**, who obtained higher GCV and heritability values coupled with high genetic advanced over mean in bitter gourd (*Momordica charantia* L.) for fruit length, average fruit weight, fruit yield per vine, fruit yield per hectar, number of seeds per fruit and fresh thickness, indicated the predominance of additive component on these traits, hence direct selection would be more effective in improving these traits.

In conclusion, genetic variability studies are important for understanding variation within species and serve as a critical starting point in crop improvement programs. High values of GCV and PCV coupled with high genetic advance were observed for plant dry weight, plant fresh weight, root length, number of nodules developed per plant, leaf area, chlorophyll a and chlorophyll b. Thus, these traits were controlled by additive gene effects leading phenotypic selection would be effective based upon phenotypes after selection cycle using the 5% selection intensity. Further, the traits having high heritability coupled with the highest genetic advance leading breeders can expect a reliable response through selection underlying these traits.

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Conflict of interest

The author declare that no conflict of interest exist.

Ethical approval

This study does not indicate any human or animal testing or feeding on the products resulted from any mutageneic treatment.

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