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CORRELATION COEFFICIENT AND REGRESSION ANALYSIS OF SOME ECONOMIC TRAITS IN GARDEN PEAS AFFECTED BY CHEMICAL AND PHYSICAL MUTAGENS

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ABSTRACT

Garden pea (Pisum sativum L.) is one of the most vegetable legume crops grown in Egypt through the winter season. In order to develop high-yieldinggenotypes in this legume crop available genetic wealth is very essential. Therefore, genetic variability induced by mutagenic agents in plant growth traits and their contribution towards seed yield may be used as a criterion for yield improvement in garden peas. The overall objective of this study was to estimate the extent of association between plant growth traits with the doses of mutagenic agents through correlation and regression analysis. One germplasm of garden pea, as well as seven doses of mutagenic agents in addition to the control, were used in this study. The experiment was carried out in a randomized complete block design with three replications in the Agri-Farm of Genetic Department inside the campus of Mansoura University. Analysis of the correlation coefficient revealed that the amount of correlation coefficients appeared very close between plant fresh weight, chlorophyll b with the mutagenic doses. Meanwhile, plant dry weight, leaf area developed per plant andtotal chlorophyll concentration exhibited a positive correlation with mutagenic doses that induced new genotypes in garden peas. Therefore, these traits may be considered the most reliable traits that respond to mutagenic agents which can be used as indicators for new genotypes induced which related to effective improvement in the growth and yield of garden peas through mutation breeding.

Keywords: Acridine Orange, Correlation Coefficient, Growth Traits, Pisum Sativum L., Regression Analysis, Relative Increase, Ultraviolet Rays.

1. INTRODUCTION

Pea (*Pisum sativum* L.) belongs to the family Fabaceae, also called garden pea, growing virtually worldwide for its edible seeds.All the cultivated and wild types of the genus *Pisum*havethe same diploid chromosome number of 2n = 2x = 14.It is also high in lysine as an important amino acid which was absent in cereals **(Yarnell 1962)**. Peas probably originated in South Western Asia, Pakistan, Afghanistan, possibly North Western India,and adjacent areas of the former USSR and thereafter spread to the temperature Zones of Europe before BC **(Mohanty *et al.* 2020)**. Green peas are consumed as vegetable, as well as marketed fresh vegetable or in soup or proceed canned or dehydrated forms or frozen **(Davies *et al.* 1985)**. Pea seeds are of two types, among them wrinkled seeded garden peas are sweeter than smooth-seeded types **(Mohanty *et al.* 2020).** Pea was grown worldwide in around 6.51 million hectares area with annual production of 10.95 million tonnes and a productivity of 12.65 t/ha. India contributes

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around 21 percent of worldwide peas production from an area of about 554 thousand hectares with an average production of 5524 thousand metric tones **(Mohanty *et al.* 2020)**.

Pea is aleguminous crop thatfixes atmospheric nitrogen through their root nodules. Nitrogen fixation reduced the use of chemical fertilizers like urea and ammonium nitrate **(Verma *et al.* 2021)**. As the most important economic crop in Egypt, its genetic development has still remainedslow.Farmers must face the lower productivity and quality in yield potential of subsisting varieties. Therefore, the main objective for the breeders is to increase yield, as well as, productivity per unit area to achieve the demand throughout the year. Yield was affected by a number of genetic factors that interact with the environmental conditions.Therefore, the success of any breeding program was determined by the availability of genetic diversity in the original population in addition to selection efficiency **(Kumari *et al.* 2008)**. Pea has a prime position in Egypt among various green vegetables because of its diverse uses.Most of the cultivated table area of peas is concentrated around the cities or near the marketing places where human labor for picking green pods, irrigation facilities and transportation facilities are available.Such facilities help the farmers collect theready pods for transportation and marketing to get higher returns **(Verma *et al.* 2021)**.

Selection is a usefulguide for plant breeders because it can be improved further by learning about the correlations between different economic traits. Correlation studies help in determining the interrelationship between different plant traits.Simple correlation provides mutual association between two variables but they do not give information about the cause and effects.If correlation studies involve many characters, then the direct association becomes more complex **(Verma *et al.* 2021)**. The correlation coefficient is a statistical technique that is used to determine the degree and direction of the relationship between two or more variables.Correlation determines the component traits on which selection can be exercised for genetic improvement in yield.It has widely used to identify economic traits that have been significantly influencedyield for potential use in selection **(Mohanty *et al.* 2020)**. Regression analysis is an important statistical tool for the analysis of genetical data. It enables the plant breeders to identify the relationships among multiple factors.Regression analysis allows for investigating the relationship between variables**(Montgomery *et al.* 2012)**.The variables arelabeled with dependent and independent.The independent variable is an input or driver factor that has an impact on a dependent variable (called outcome).Therefore, the power of regression analysis was to determine the likelihood of increased success is influenced by factors as doses of mutagenic agents, andtype of mutagen as physical or chemical mutagens.There are generally three types of regression and analyses namely multiple, linear and logistic regression.Multiple regression examines the relationship between one or more independent and dependent variables.Meanwhile, linear regression examines the relationship between one independent variable with one dependent continuous variable.However, logisticregression calculates the likelihood of an event with a binary outcome **(Ali and Younas 2021)**. In this study linear regression analysis was used to examine therelationship between the doses of chemical and physical mutagens as an independent variable with one dependent continuous variable of morphological traits affected by mutagenic agents.

Ultraviolet rays UV is electromagnetic radiation have wavelengths ranging from 10-400 nanometers, shorter than visible light, but longer than X-rays.It was produced from Cherenkov radiation, electric arcs and specialized lights such as mercury-vapor lamps, black lights and tanning lamps. Ultraviolet has the minimum energy required to ionize atoms greater than those

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of visible light, from about 3.1 to 12 electron volts.Shortwavesof ultraviolet light are ionizing radiation.The biological effects of UV radiation are derived from the way of interaction with organic molecules in the cell.Consequently, the shortwave of ultraviolet rays damages DNA and sterilizes the surface with which it contacts. Most of the UV radiation present in sunlight is filtered out by the atmosphere **(Haigh 2007)**. The long wavelength of UV was not considered as ionizing radiation because its photons lack enough energy. The long wavelength of ultraviolet rays can induce chemical reactions. Ultraviolet rays specifically UV-B is responsible for the generation of vitamin D in most vertebrates living on the land including humans **(Wacker and Holick 2013)**. Ultraviolet radiation was first discovered in 1801 when the German physicist Johann Wilhelm Ritter found that the violet end of the visible spectrum darkened silver chloride-soaked paper more quickly than the violet light itself. He named them de-oxidizing rays to emphasize chemical reactivity, as well as to distinguish them from heat rays. The term heat rays were eventually dropped in favor of infrared radiation **(Beeson and Mayer 2007)**. Sunlight on the top of Earth's atmosphere was consists of about50%infrared light, 40% visible light and 10% ultraviolet light from a total intensity of about 1400 W/m^2 in vacuum **(Bark *et al.* 2000)**. At the ground level when the sun is highest in the sky,sunlight containing 44% divisible light,3% ultraviolet and the remainder infrared **(Bolton and Colton 2008)**. More than 95% of ultraviolet rays reach the Earth's surface in the longer wavelengths of UVA with the small reminder of UVB. Almost none of the UVC reached the Earth's surface **(Calbó*et al.* 2005)**. The ozone layer is especially important in blocking almost UVB, as well as the remaining part of UVC not already blocked by oxygen in the air **(Sivamani *et al.* 2009)**. In the past, UVA was considered less harmful than UVB, but today it is well known to cause indirect DNA damage through free radicals formed as reactive oxygen species and hydroxylradicals, which inturncan damage DNA. The DNA damage caused indirectly by UVA contains mostly single-strand breaks in DNA, meanwhile, the direct damage of UVB includes the formation of thymine dimers or cytosine dimers, as well as double-strand DNA breakage **(Svobodová *et al.* 2012)**. Most pyrimidine dimers induced in DNA by ultraviolet radiation are removedby nucleotide excision repairthat includes about 30 different proteins **(Bernstein *et al.* 2002)**. Some pyrimidine dimers that escape from nucleotide excision repair can induce a form of programmed cell death (apoptosis) or can cause errors in DNA replication leading to mutation **(Chang 2020)**.

Acridine orange is an organic compound, useful for cell cycle determination because it serves asa nucleic acid-selective fluorescent dye with cationic properties. Acridine orange interactswith DNA or RNA through electrostatic attraction by intercalation because it is cell-permeable. It is able to cause low pH environments, leading the fluorescent dye to penetrate acidic organelles such as lysosomes and phagolysosomes. The ability to penetrate cell membranes of acidic organelles allows acridine orange to differentiate between different types of cells **(Yektaeian *et al.* 2019)**. Acridine orange staining was performed at acidic pHto producedifferential staining leading to bacterial cell stain orange and tissue components staining yellow or green **(Darzynkiewicz *et al.* 2004)**. Acridine orange was used as a curing agent to cure selectable markers from antibiotic-resistantorganisms to be cured of at least one resistant marker.Acridine orange emits red fluorescence when RNA binds by sticking interactions and green fluorescence when DNA binds by intercalation **(Sharma *et al.* 2020)**. Keeping these in view, the current study was undertaken to elucidate the magnitude of correlations between growth and biochemical traits with the doses of chemical and physical mutagens in order to find indicators that could be used for improving growth under abiotic stress.In addition, regression

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analysis was performed in this study to predict the variation in growth parameters labeled as a dependent variable named outcome based on mutagenic doses, labeled as an independent variable input.

2. MATERIALS AND METHODS

This study was carried out in the Experimental Research Agri-Farm of Genetic Department, Faculty of Agriculture, Mansoura University, Egypt during the academic year of 2022/2023. The Agri-farm is located inside the campus of Mansoura University.

Genetic material

The experimental material comprised one genotype of garden pea, *Pisum sativum* L. named Almomtaz. This genotype was kindly received from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt.

Ultraviolet rays

The UV lamp in the laminar cabinet located in the Laboratory of Microbial Genetics, Faculty of Agriculture, Mansoura University, was used for irradiated the seeds of garden pea, *Pisum sativum* L. The spectrum of this lamp was 300 nm, leading to be classified as UV-B **(Barta *et al*. 2004).**

Acridine orange

Acridine orange is able to penetrate acidic organelles membranes in the cell as lysosomes **(Narjes *et al*. 2019)**. It is an organic compound that serves as a nucleic-acid selective fluorescent dye. Acridine orange interacts with DNA by intercalation or RNA with electrostatic attractions. Preferred IUPAC name N, N, N, N-tetramethylacridine- 3,6-diamine **(Yektaeian *et al*. 2019).**

Mutagenic treatment

The seeds were treated with the 200 ppm acridine, 15, 30 and 45 minutes of exposure time to ultraviolet irradiation, in addition to the interaction between physical and chemical mutagen as shown in treatment details furnished in **Table 1**. About 100 seeds were treated with each dose of chemical and physical mutagens and the interaction between both of them. The seeds were immersed in tap water for six hours and then soaked in acridine solution for another six hours before being exposed to different doses of UV irradiation. Soaked seeds in tap water only for six hours were served as a control.

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Table 1. Treatment details.

Treatments	Mutagen (chemical/physical)	Concentration/Dose
T_1	Untreated control	0.0
T_2	Ultraviolet rays (UV)	15 minutes
T_3	Ultraviolet rays (UV)	30 minutes
T_4	Ultraviolet rays (UV)	45 minutes
T_5	Acridine orange (ACO)	200 ppm
T_6	Acridine orange (ACO) + Ultraviolet rays (UV)	200 ppm ACO + 15 minutes UV = 215
T_7	Acridine orange (ACO) + Ultraviolet rays (UV)	200 ppm ACO + 30 minutes UV = 230
T_8	Acridine orange (ACO) + Ultraviolet rays (UV)	200 ppm ACO + 45 minutes UV = 245

Table 1.Continued.

Treatments	Mutagen (chemical/physical)	Designation
T_1	Untreated control	0.0
T_2	Ultraviolet rays (UV)	15
T_3	Ultraviolet rays (UV)	30
T_4	Ultraviolet rays (UV)	45
T_5	Acridine orange (ACO)	200
T_6	Acridine orange (ACO) + Ultraviolet rays (UV)	215
T_7	Acridine orange (ACO) + Ultraviolet rays (UV)	230
T_8	Acridine orange (ACO) + Ultraviolet rays (UV)	245

Experimental set-up

The experiment was laid out in an Augmented Block Design with three blocks, each block comprising eight treatments. Each block was three meters long and 7.2 meters wide. The seeds were sown in the field trial to obtain M1 populations. The rows were three meters long and 30 cm wide. Four seeds were sown in each hole, each row containing eight holes. The seed-to-seed and row-to-row distance was conducted at 30 cm and 60 cm apart, respectively. After 15 days of emergence, the plants were carefully thinned to two healthy plants in each individual hill. Recommended agronomic practices and plant protection were carried out as in the recommended package released from the Egyptian Ministry of Agriculture. The plants were irrigated regularly when needed by river water. The observations of growth and biochemical traits were measured in M1 populations that may carry the dominant mutant phenotype. The importance of M_1 is ensuring maximum survival which is beneficial for plant breeders to obtain the seeds of M_2 generation. However, most mutations are recessive in nature, therefore they can not be observed in heterozygous states of M_1 populations. The recessive mutants were observed in the homozygous state of the M_2 generation after the seeds were developed by fusion between male and female gametes carrying the same recessive mutation **(Kumar *et al*. 2019).**

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Data collection

Data were collected on a plot basis from the central rows for all traits. For data recorded on a plant basis, three plants were randomly selected and the mean values were calculated using Microsoft Excel. Growth and biochemical traits measured in this study are listed in **Table 2**.

Table 2. List of growth and biochemical traits, as well as the techniques of evaluation.

Trait	Technique of evaluation
Plant fresh weight (g)	Three plants were randomly selected from each treatment in each plot at 50-days plant old. The plants were carefully washed and weighted.
Plant dry weight (g)	After taken the plant fresh weight, the plants were oven-dried at 70 ^{0}C until reached to the constant weight and then weighted immediately.
Root length (cm)	Root length was measured from the soil surface up to the tip of the root when the plants become to blooming according to **Shuro*et al*. (2018).**
Number of primary branches per plant	The average number of primary branches per plant from three sampled plants were counted when the plants become to blooming according to **Shuro*et al*. (2018)**.
Number of nodules per plant	The average number of nodules formed on the roots of three sampled plants at 45 days plant old were counted according to **Shahid *et al.* (2018)**.
Leaf area (cm^2)	Leaf area was measured at 50-days plant old based on ten disks taken from ten fresh leaves in each plant using cork piercing 1.5 cm diameter according to **Favarin*et al*. (2002)**.
Chlorophyll pigments (mg/g FW)	It was measured in leaves spectrophotometrically at 50-days plant old according to the method explained by **Arnon (1949)**.
Carotenoids content (mg/g FW)	The contents of carotenoids in leaves at 50-days plant old and pods were estimated spectrophotometrically according to the method explained by **Arnon (1949)**.

Yield and relative increase

Results are the mean values of three biological replicates from each sector.The data were expressed as yield in relation to control.Meanwhile, the relative increase over control was calculated according to **Garcia *et al*. (1988).**

Correlation analysis

Correlation coefficient between the traits under investigation was estimated according to the statistical techniques of **Gomez and Gomez (1984).**

Regulation analysis

The purpose of statistical evaluation of genetical data is often to describe relationships between two variables to know whether the likelihood of economictraits is influenced by the doses of mutagenic agents. The variable that explains economic traits affected by mutagens is called the

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dependent variable or alternatively the response variable,the variable that explains it as mutagenic doses used in this study is called the independent variable or predictor variable. Regression analysis is a model that describes the relationships between the dependent variable as economical traits and the independent variable as mutagenic doses in a simplified mathematical form according to **Fahrmeir *et al*. (2009)**. The linear regression model is defined by the equation,Y = a + bX, where a is the Y-intersect of the line, b is its slope, Y is the dependent variable and X is the independent variable. The regression lineenables geneticists to predict the value of dependent variable Y as economical traits from that of the independent variable X as mutagenic doses used in this study.The slope b of the regression line is called the regression coefficient.If the independent variable is continuous as economical traits, then the regression coefficient represents the change in the dependent variable as economical traits per unit of change in the independent variable as mutagenic agents. The proper interpretation of the regression coefficient requires attention to the units of measurement **(Carpenter and Kenward 2008).**

3. RESULTS AND DISCUSSION

Growth traits

As shown from the results tabularized in Table 3 the genetically diverse of M1 population appeared positive relative increase in plant fresh weight ranging between + 3.74 to + 70.09%. The highest relative increase in plant dry weight (+ 70.09%) was achieved in response to the treatment with 200 mg ACO + 30 min UV. Meanwhile, the lower relative increase in plant dry weight (+ 3.74%) was achieved in response to 200 mg ACO + 45 min UV. These results indicated that the plants affected by acridine or the interaction between acridine and ultraviolet irradiation may form a higher number of leaves per plant in addition to lower evaporation of water from the plant tissues. It is noticed from the Table that 200 mg ACO still increased plant dry weight over the control by + 3.82%. In addition, the interaction between acridine and ultraviolet rays continued to increase plant dry weight over the control by + 51.59%. These results agreed with Sekhi et al. (2021), who found that the acridine mutagen levels produced the highest concentration of leaves number per plantlet of strawberry ranked after control. The relative increase in root length over the control was achieved with all the mutagenic doses except for at 15 min exposure to ultraviolet irradiation. The relative increase in root length ranged between + 12.98% to + 87.50%.

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Table 3. Yield and relative increase percent of different growth traits in garden peas responded to various doses of chemical and physical mutagens.

Doses of mutagens	Plant fresh weight(g)		Plant dry weight(g)		Root length(cm)		Number of branches/plant		Number of nodules/plant		Leaf area(cm^2)	
	Yield %	RI %	Yield %	RI %	Yield %	RI %	Yield %	RI %	Yield %	RI %	Yield %	RI %
0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
15 min UV	58.88	-41.12	87.26	-12.74	94.52	-5.48	87.50	-12.50	68.62	-31.28	81.39	-18.61
30 min UV	66.35	-33.65	77.07	-23.93	137.50	+37.50	71.25	-28.75	48.83	-51.17	47.97	-52.03
45 min UV	73.83	-26.17	47.13	-52.87	187.50	+87.50	116.25	+16.25	22.96	-77.04	97.22	-2.78
200 mg AC	138.32	+38.32	103.82	+3.82	114.42	+14.42	71.25	-28.75	94.57	-5.43	76.75	-23.25
200 mg AC + 15 min UV	134.58	+34.58	5.09	-94.91	136.15	+36.15	75.00	-25.00	61.48	-38.52	94.93	-5.07
200 mg AC + 30 min UV	170.09	+70.09	151.59	+51.59	123.36	+23.36	91.25	-8.75	47.56	-52.44	133.59	+33.59
200 mg AC + 45 min UV	103.74	+3.74	80.89	-19.11	112.98	+12.98	100.00	0.00	59.67	-40.33	34.83	-65.17

RI = Relative increase.

The highest increase in root length (+ 87.50%) was obtained from the plants irradiated with 45 min UV, meanwhile, the lower increase in root length (+ 12.98%) was achieved from the plants treated with 200 mg ACO + 45 min UV. These results indicated that the mutagenic effect of acridine orange and ultraviolet rays leads to increased root length through the stimulation of cell division and cell elongation. This may be due to the mutations induced which affect not only the physiological traits but also the anatomical integrity of the plant (Castronuovo et al. 2015). These results agreed with Hopkins et al. (2002), who decided that the reduction in plant growth under the stress of UV resulted in an alteration in the rate of cell division and elongation, this may be due to the inhibition of indole acetic acid (IAA) as a key regulator of plant growth. The same authors reported that oxidation induced in IAA by UV irradiation may be related to increased axillary branching (Meijkamp et al. 2001). The increase obtained in root length may be due to the efficiency of high carbon assimilation capacity via photosynthetic rates increased (Kumari et al. 2009). The dose of 45 min UV is the only dose of mutagens induced relative increase over the control in the number of branches developed per plant reached + 16.25%. All doses of mutagenic agents used in this study showed negative effects on the nodulation process. The treatment with 200 mg ACO + 30 min UV is the only treatment of mutagenic agents that produced a relative increase over the control in leaf area developed per plant reaching + 33.59%. Increasing leaf area leading the greatest plant biomass. Therefore, the dose of 200 mg ACO + 30 min UV appeared to relatively increase over the control in plant fresh weight, plant dry weight, root length, as well as leaf area. These results agreed with Garcia et al. (1988), who found that inoculated plants with Rhizobium induced the greatest plant biomass and nodule weight. The results suggested that the volume of leaf area was able to support optimum dry matter accumulation. It should be noted that under the effect of interaction between chemical and physical mutagens (200 mg ACO + 30 min UV), the plants showed better growth in plant fresh weight, plant dry weight, root length and leaf area developed per plant. Meanwhile, the treatment with 200 mg ACO + 45 min UV showed the lowest relative increase over the control in plant fresh weight and root length. Mutagenic agents interact with the oxygen atom, nitrogen and carbon in the nitrogen bases were affecting on the genes encoding protein biosynthesis and thus affects the growth characteristics (Sahasrabudhe et al. 1991). The results indicated that mutagenic agents have a positive effect on the development of traits especially root length which showed a relative increase over the control at most doses of mutagens. The molecular level of a plant adopts itself by activating genes leading to manufacturing defensive metabolites (Muchate et al. 2016). Acridine orange was attributed to increasing the activity of the oxidase enzyme NADPH resulting from stimulating the production of reactive oxygen species within the plant vegetable part (Zhang et al. 2018).

Chlorophyll pigment

The results of yield and relative increase over the control in chlorophyll piments (Table 4) indicated that the dose of 30 min UV showed the highest relative increase in total chlorophyll concentration (+ 82.76). This increase was attributed to the relative increase in chlorophyll a, as well as in chlorophyll b. The relative increase in total chlorophyll ranged between +27.58 to +80.76% mainly due to the increase in chlorophyll b concentration. This indicated that chlorophyll b concentration has the main influence on the relative increase in total chlorophyll concentration.

Table 4. Yield and relative increase percent of different biochemical traits in garden peas responded to various doses of chemical and physical mutagens.

Doses of mutagens	Chlorophyll in leaves(mg/g FW)						Carotenoid (mg/g FW)			
	a		b		Total		leaves		pods	
	Yield %	RI %	Yield %	RI %	Yield %	RI %	Yield %	RI %	Yield %	RI %
0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00
15 min UV	+42.86	-57.14	93.33	-6.67	68.97	-31.03	76.25	-23.75	80.00	-20.00
30 min UV	+107.14	+7.14	253.33	+153.33	182.76	+82.76	83.75	-16.25	75.38	-24.62
45 min UV	+7.15	-92.85	73.33	-26.67	41.38	-58.62	87.50	-12.50	87.46	-21.54
200 mg AC	+21.43	-78.57	266.67	+166.67	151.72	+51.72	79.37	-20.63	90.76	-9.24
200 mg AC + 15 min UV	+14.28	-85.72	233.33	+133.33	127.58	+27.58	72.50	-27.50	64.61	-35.39
200 mg AC + 30 min UV	+28.57	-71.43	260.00	+160.00	148.27	+48.27	98.12	-1.88	72.31	-27.69
200 mg AC + 45 min UV	28.57	-71.43	273.33	+173.33	155.17	+55.17	88.12	-11.87	55.38	-44.62

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The doses of 200 mg ACO, 200 mg ACO + 15 min UV, 200 mg ACO + 30 minUV and 200 mg ACO + 45 minUV revealed a relative increase over the control in total chlorophyll concentration. These findings are well-matched with the results of Nasim and Brychcy (1979), who found that acridine orange caused chromosomal abnormalities and mutations inchlorophyll, as well as phenotypic mutations. Meanwhile, Azooz et al. (2012) found that Cu supplementation at toxic levels induces ROS production in chloroplast and elevated catalase activities in wheat. The catalase enzyme is responsible for the decomposition of H2O2 stored in the cell peroxisomes (Emrahi et al. 2021).

Carotenoids were decreasedin leaves and pods with different percentagesranging between -1.88% to -27.50% in leaves and -9.24% to -44.62% in pods. The highest decrease of carotenoids in leaves (-27.50%) occurred at 200 mg ACO + 15 minUV,whereas the lowest decrease was shown at 200 mg ACO + 30 minUV.On the other hand, the highest decrease of carotenoids in pods (-44.62%) happened in response to 200 mg ACO + 45 minUV,meanwhile,the lowest decrease (-9.24%)occurred at 200 mg ACO. These results agreed with Behtash et al. (2022), who found that the values related to chlorophyll a and b content in summer squash were substantially decreased along with increased Cu concentration if compared with their respective control. At the same time, Behtash et al. (2022)found that carotenoid content increased when the plants of summer squash were exposed to increased Cu concentration. Rodriguez et al. (2018) decided that under copper stress predicament, carotenoids are the first-line defenders against oxidative damage to chlorophyll.The results obtained in this study declared that mutagens stress reduced the expression of some genes involved in carotenoid biosynthesis and carbon assimilation. The mean comparisons of Behtash et al. (2022)revealed that the main effectsdepicted that Zn application increased the concentration of chlorophyll a, b and total chlorophyll, but decreased carotenoid concentration.Therefore, the same authors reportedthat Cu supplementation achieved the reverse behavior if compared withZn. Therefore,mutagenic agents used in this study affected the gene expression regulation encoded carotenoid in a parallel line with protein expression by enhancing RNase activity as mRNA degrading enzymes (Umair Hassan et al. 2020). The results mentioned above can be interpreted in this sense that the boosted suppression of antioxidant enzymes protective carotenoids under the effects of mutagenic agents is possibly due to the decreased expression of these proteins through enough support of mutagens. In this respect, Emrahi et al. (2021) reported that carotenoids and chlorophyll b play a significant protective role through scavenging singlet oxygen (O2), as well as anion superoxide (O-2), which damage the reaction centers in photosynthetic complexes. This study confirmed that the increased concentration of mutagens can result in an intense depression of carotenoid concentrations in leaves and pods which leads the plant cell to be more sensitive to mutagenic agents. Acridine orange binds with sulfur compounds inside the plant cells which is considered a cationic compound (Schrawat et al. 2016). The binding of acridine with the seventh nitrogen atom in the guanine nitrogen base is the site of its main effects (Kresty et al. 2001). It may also lead to morphological changes in DNA which resulted in unspecified cloning of a number of genes as carotenoid-forming genes. The mechanism of the binding process of acridine orange with DNA is strong, because one molecule of acridine may bind with four nitrogen bases. Therefore, acridine orange caused chromosome breakage in the legumes (Ulitzur and Weiser 1981).

Correlation analysis

As shown from the results tabulated in Table 5 there were a significant correlation between total chlorophyll and chlorophyll b.

Table 5. Correlation coefficient among different growth and biochemical traits in garden peas influenced by chemical and physical mutagens.

Traits	Plant fresh weight	Plant dry weight	Root length	Number of branches/ plant	Number of nodules/ plant	Leaf area	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid in Leaves	Carotenoid in Pods
Plant fresh weight	1.00	0.33 ^{NS}	-0.13 ^{NS}	-0.23 ^{NS}	0.19 ^{NS}	0.51^{NS}	-0.39^{NS}	0.59^{NS}	0.42 ^{NS}	0.22^{NS}	-0.11^{NS}
Plant dry weight		1.00	-0.46^{NS}	0.03^{NS}	0.25^{NS}	0.27^{NS}	0.24^{NS}	0.18^{NS}	0.27^{NS}	0.64^{NS}	0.32^{NS}
Root length			1.00	0.35^{NS}	-0.79*	0.12^{NS}	-0.35^{NS}	-0.15^{NS}	-0.28^{NS}	-0.06^{NS}	-0.21^{NS}
Number of branches/plant				1.00	-0.37^{NS}	0.21^{NS}	-0.21^{NS}	-0.61^{NS}	-0.67^{NS}	0.55^{NS}	-0.002^{NS}
Number of nodules/plant					1.00	-0.05^{NS}	0.33^{NS}	0.06^{NS}	0.20^{NS}	0.03^{NS}	0.58^{NS}
Leaf area						1.00	-0.24^{NS}	-0.30^{NS}	-0.38^{NS}	0.35^{NS}	0.34^{NS}
Chlorophyll a							1.00	-0.08^{NS}	0.30^{NS}	0.35^{NS}	0.42^{NS}
Chlorophyll b								1.00	0.92**	-0.11^{NS}	-0.49^{NS}
Total Chlorophyll									1.00	0.02^{NS}	-0.29^{NS}
Carotenoid in Leaves										1.00	0.28^{NS}
Carotenoid in Pods											1.00

NS = Not significant ** = Significant at 0.01 level of probability.

Thus, it can be concluded that higher concentrations of total chlorophyll were more affected by the concentration of chlorophyll b. A positive correlation was also obtained between plant fresh weight with plant dry weight, plant dry weight with the number of branchesdeveloped per plant, root length with number of branches developedper plant, plant fresh weight with a number of nodules developedper plant, plant dry weight with number of nodules developedper plant. In addition, a positive correlation was obtained between leaf area developed per plant with the following traits; plant fresh weight, plant dry weight, root length and number of branches developed per plant.

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Meanwhile, a positive correlation was obtained between chlorophyll a with plant dry weight and the number of nodules developedper plant. A positive correlation was also obtained between chlorophyll b with plant fresh weight, plant dry weight and number of nodules developedper plant. Total chlorophyll concentration showed a positive correlation with plant fresh weight, plant dry weight, number of nodules per plant, chlorophyll a and chlorophyll b. On the other hand, the carotenoid concentrations in leaves achieved a positive correlation with plant fresh weight, plant dry weight, number of branches developedper plant, number of nodules developedper plant, leaf area, chlorophyll a and total chlorophyll. In addition, the carotenoid concentration in pods was positively correlated with plant dry weight, number of nodulesdeveloped per plant, chlorophyll a and carotenoid concentration in leaves. These results agreed with **Guleria *et al*. (2009)**, who decided that a positive correlation between desirable traits would be preferred by the plant breeders to be going for simultaneous improvement of the correlation traits. Meanwhile, a negative correlation between desirable traits is of immense scope in plant breeding. The correlation coefficient indicates the relationship existing between two variable traits. **Nawab *et al*. (2008)** found similar findings in pea. Meanwhile, a dependent variable such as seed yield per plant, for example, is the product of interaction between different mutually associated traits, therefore the change in any one component will disturb the whole network of the cause and influence the system. This will be helpful in breeding strategies in evolving efficient selection for reducing the negative effects and maximizing the synergistic effects. The increase in thenumber of components becomes a complex process. Correlation analysis gives better insight into the relationship between different economical traits. The results obtained herein agreed with **Tarkeshwar *et al*. (2020)**, who reported that selection based on growth traits could definitely leadto improving grain yield. The results are also in harmony with **Fikre *et al*. (2012)**, who found a positive significant association between grain yield of groundnut with pod dry yield, total of pods per plant, 100-seed weight, number of branches per plant and oil content. Correlation coefficient helps to describe the interaction between economic traits by providing a symmetrical value about the degree of interaction between two variables. It described the importance of understanding the interactions between one variable and related variables as a requirement for generating a successful selection program with the goal of increasing seed yield **(Pratap *et al*. 2021).** In the earlier studyof **Parihar *et al*. (2014)**, who found that grain yield per plant in *Pisum sativum* var *arvense* L. was significantly positively correlated with the number of primary branches per plant, plant height and number of pods per plant. The selection practiced for employing growth traits individually or simultaneously would bring the improvement of others due to correlated responses. Therefore, selection will be efficient for the traits that are major yield contributing traits. In addition, the traits exhibit a positive correlation towards the other traits leading selection based on these traits will be worthwhile for any breeding program. So results would help the plant breeder to select high growth through selection for one or more of these traits. This finding agreed with **Pavan *et al*. (2011)**, who recorded a significant positive phenotypic correlation between grain yield in maize and plant height. On the other hand, **Virk and Anand (1970)** found that grain yield in wheat was positively and significantly correlated with spike length, biomass yield per plot and 1000-grain weights, which support the present study.

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Regression analysis

According to **Figure 1,** plant fresh weightexhibited a significant positive association with the doses of mutagenic agents (r = 0.795). The regression coefficient of 0.0309, in this model, means that plant fresh weight was predicted to increase by 0.0309 g with each additional unit of mutagenic agents inside the Figure. The constant a, in contrast, is independent of the unit chosen to express the independent variable.

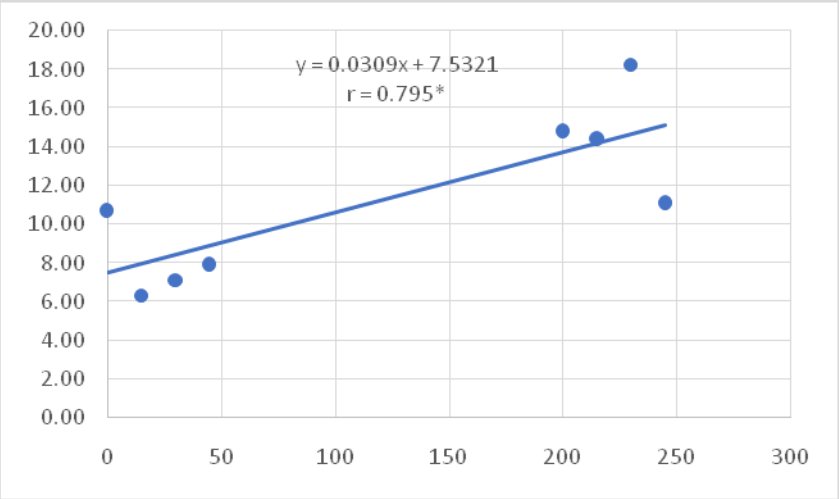

Figure 1. Regression line of response variable plant fresh weight belongs on the y-axis against explanatory variable the mutagenic doses belongs on the x-axis.

The regression coefficient should be considered together with the units used with all of the involved variables. Linear regression could be used to estimate the predicted plant fresh weight with any dose of mutagenic agents lies within the observed range (0-250). Mathematically, it is possible to estimate plant fresh weight with the doses is outside the range of mutagenic doses used in this study. Such an extrapolation is generally not useful.

Regarding to **Figure 2** the association between plant dry weight and mutagenic doses was an insignificant positive correlation (r = 0.071). The regression coefficient of 0.0004 means that plant dry weight was predicted to increase by 0.0004 g with each additional unit of mutagenic agents located in this Figure. This indicated that the response variable plant dry weight depends on the independent variable (mutagenic agents).

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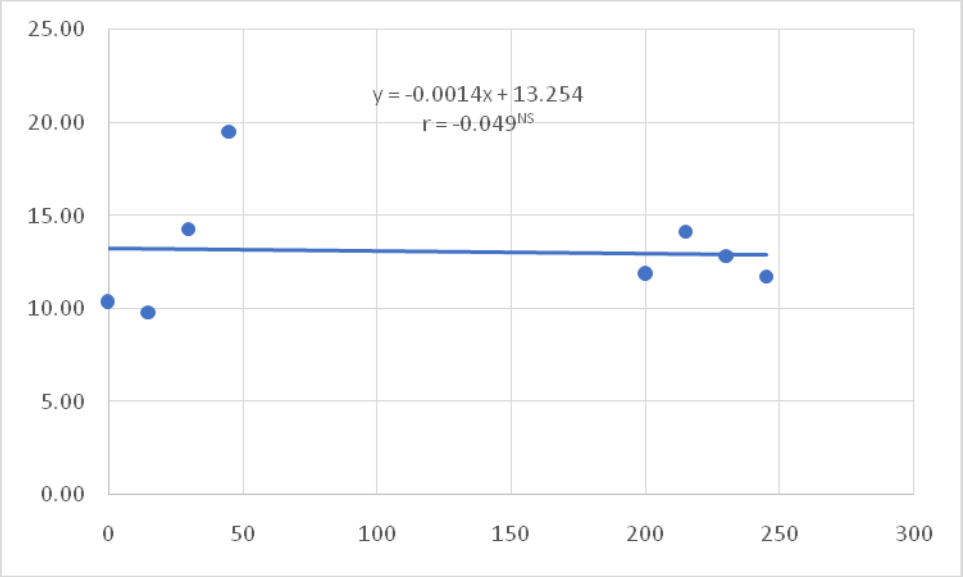

Figure 2. Regression line of response variable plant dry weight belongs on the y-axis against explanatory variable the mutagenic doses belongs on the x-axis.

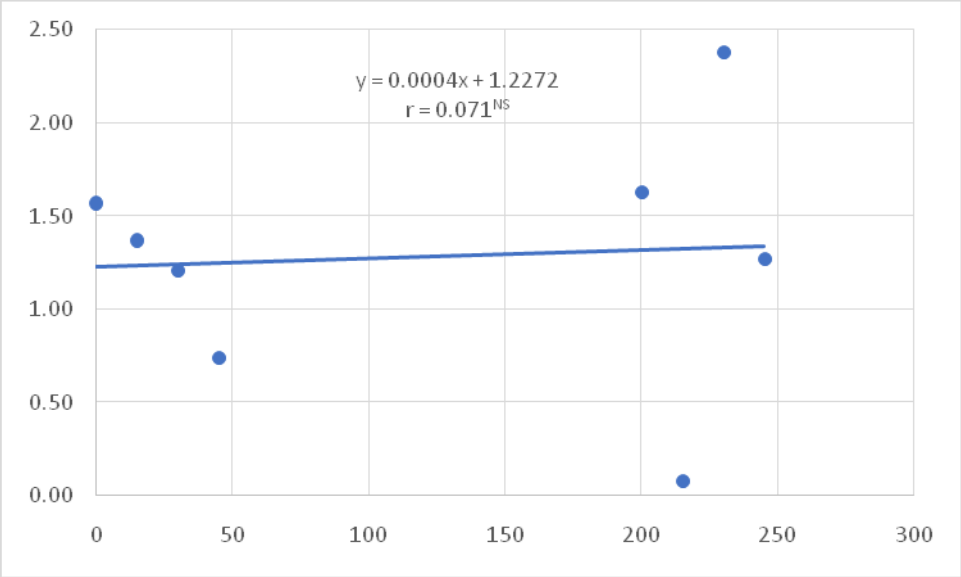

Figure 3. Regression line of response variable root length belongs on the y-axis against explanatory variable the mutagenic doses belongs on the x-axis.

As shown from the results presented in **Figure 3**, it is already revealed that there was an insignificant negative correlation (-0.049) between root length and mutagenic doses. The regression coefficient obtained between the response variable (root length) and the explanatory

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variable (mutagenic doses) of – 0.0014 means that the root length was predicted to decreased by – 0.0014 with each additional unit of mutagenic agents. This describes how a response variable root length changes as an explanatory variable mutagenic agents change. This regression line can be used to predict the value of root length for a given dose of mutagenic agents. On the other hand, this regression line shows how much and in what direction the root length as a response variable changes if the explanatory variable mutagenic agents changes.

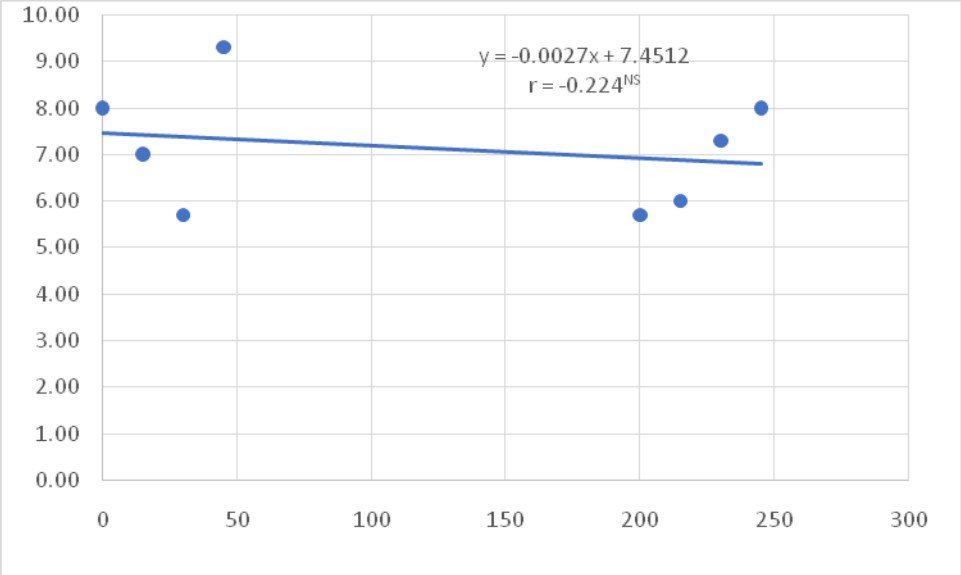

Figure 4. Regression line of response variable number of branches developed per plant belongs on the y-axis against explanatory variable the mutagenic doses belong on the x-axis.

According to **Figure 4** a negative association (r = -0.224) was obtained between the number of branches developed per plant with the doses of mutagenic agents. This indicated that the relationship between both variables was negative. If the independent variable mutagenic doses increased, then the response variable number of branches developed per plant was decreased, because the mutagenic agents were affected to decrease IAA which is necessary for the development of primary branches. The regression coefficient between the number of branches per plant with mutagenic doses of - 0.0027 meansin this model that the number of branches per plant was decreased by – 0.0027 with each additional unit of mutagenic agents. The coefficient of determination (r) is a measure of how well the regression line described the observed data. The regression line closely approximates all the points in the Figure.

The results diagrammatic in **Figure 5** exhibited an insignificant positive association (r = 0.026) between the number of nodules formed per plant with mutagenic doses. A positive value of association between these two variables shows that the changes of both variables are in the same direction. This means that the high value of one variable was associated with the high values of the other variable and vice versa. Therefore, the plant breeder was always concerned with the selection of suitable doses that induced superior genotypes on the basis of phenotypic expression of the trait under investigation. In the quantitative traits, genotypes induced by chemical mutagens are influenced by environmental factors, thereby affecting the phenotypic expression.

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Information about the nature and association between morphological traits with mutagenic doses would be helpful in developing a mutation breeding technique to induce a suitable plant genotype, in addition, to improving the yield-related traits as a complex trait for which direct selection is not effective. These results are in harmony with **Singh *et al* (2022)**, who found that day to 50 percent flowering registered a positive significant association with the days to first harvest on the genotypic and phenotypic levels. The regression coefficient of – 0.0033 means that the number of nodules developed per plant was decreased by – 0.0033 with each additional dose of mutagenic agents. The purpose of regression line herein was to predict the direction between the number of nodules developed per plant and mutagenic agents. Therefore, b is the slope meaning that the amount by which nodule number changed for every one unit increase in mutagenic doses.

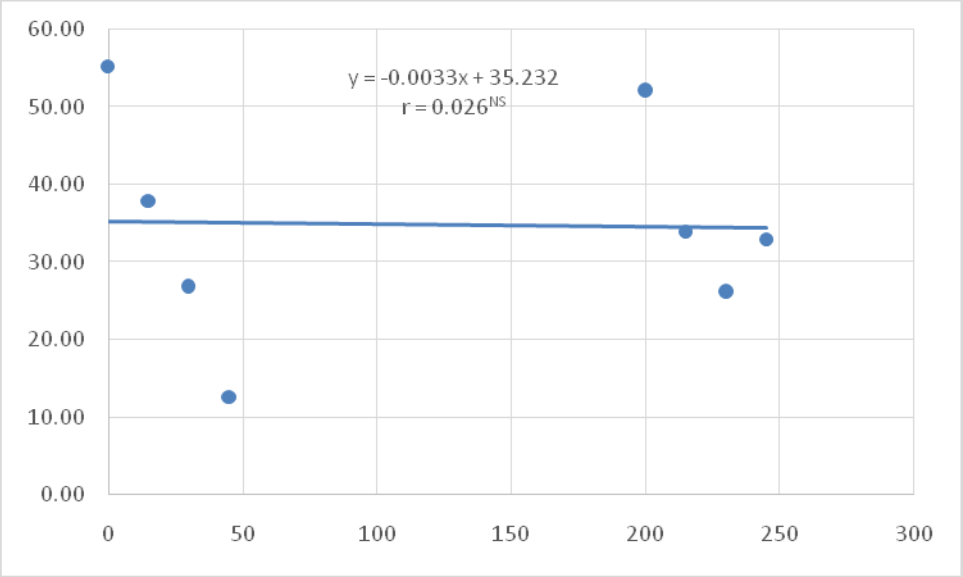

Figure 5. Regression line of response variable number of nodules developed per plant belongs on the y-axis against explanatory variable the mutagenic doses belong on the x-axis.

The regression line present in **Figure 6** appeared insignificant positive correlation (r = 0.016) between the leaf area developed per plant with mutagenic doses which indicates that the changes in both variables are in the same direction. The regression coefficient between both variables of 0.0222 meaning that the leaf area developed per plant increases by 0.0222 with each additional unit of mutagenic agents inside the Figure. In the particular fictitious case described herein, the coefficient of determination (r) concerning the relationship between leaf area and mutagenic doses is 0.016. This means that r^2= 0.000256, therefore, approximately 0.0256 % of the variance in leaf area is due to mutation induced in the garden pea genotype. The remaining 99.97% of the variance might be explained by other factors that were not taken into account in the analysis.

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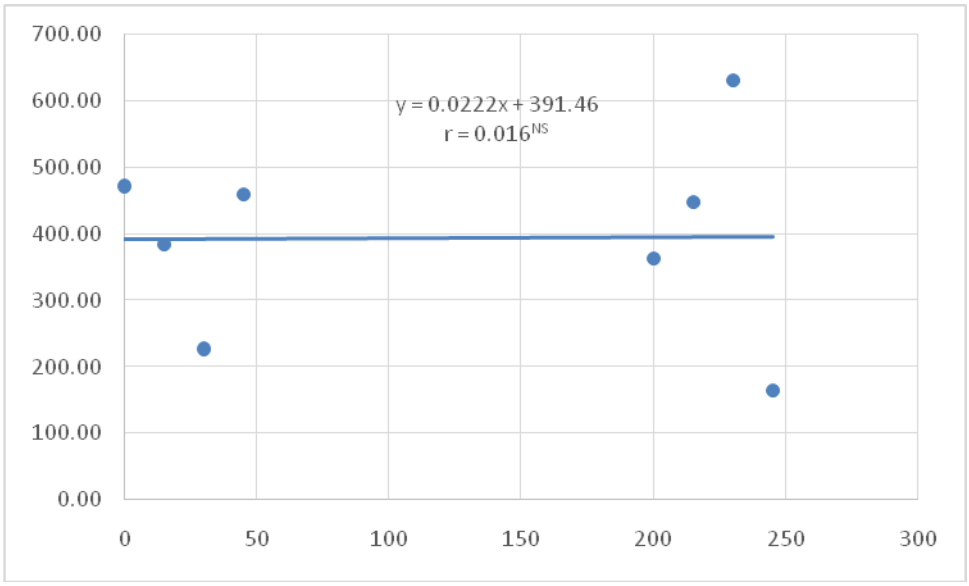

Figure 6. Regression line of response variable leaf area belongs on the y-axis against explanatory variable the mutagenic doses belong on the x-axis.

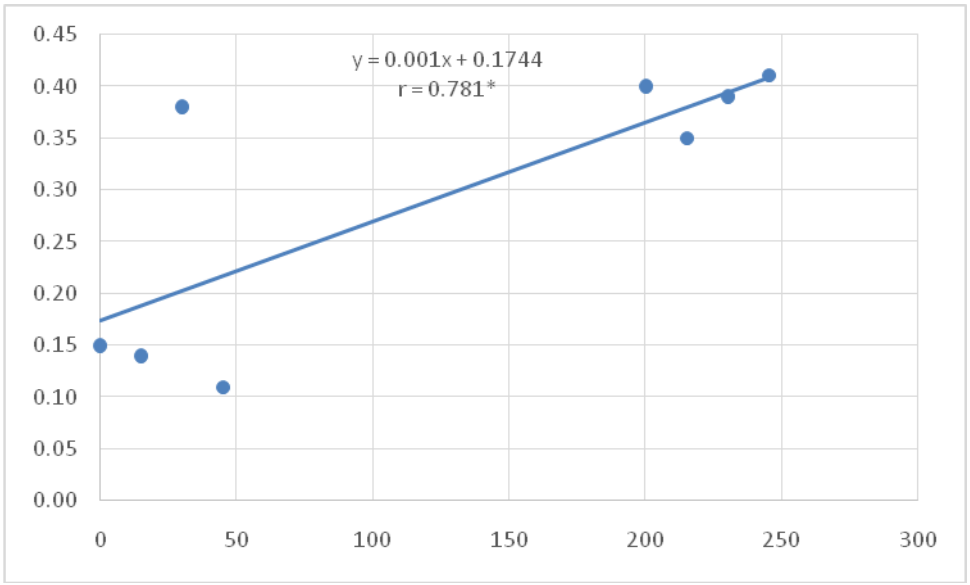

Figure 7. Regression line of response variable chlorophyll a belongs on the y-axis against explanatory variable the mutagenic dose belongs on the x-axis.

Estimate of correlation coefficient between chlorophyll a in leaves with mutagenic doses **(Figure 7)** exhibited insignificant and negative correlation (-0.609) between both variables. The coefficient of determination in this relationship means that the changes of one variable are vice

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versa with the changes in the other variable. This indicated that the high values in chlorophyll a are not associated with the high doses of mutagenic agents. The regression coefficient of – 0.0003 means that chlorophyll a formation decreased by – 0.0003 with each additional unit of mutagenic agents.

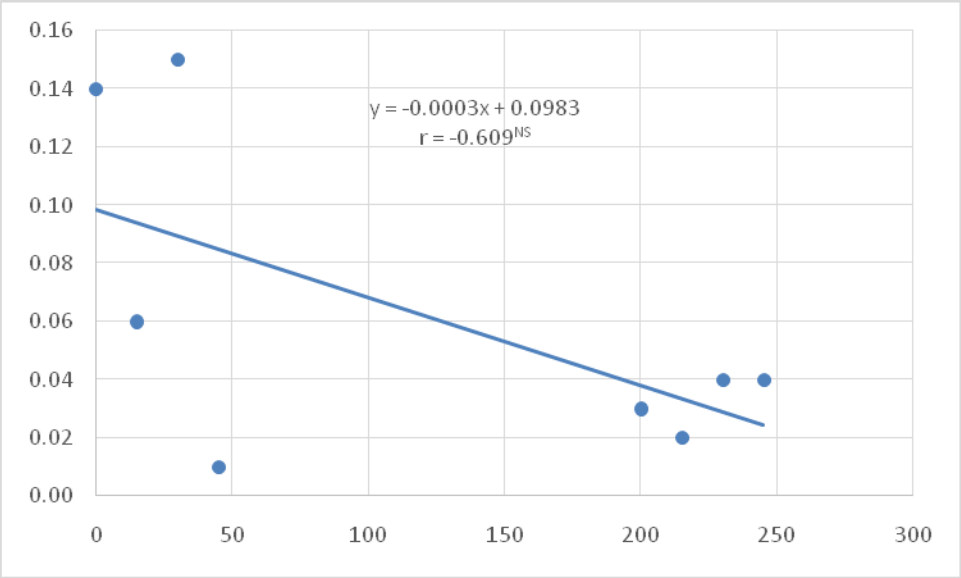

Figure 8. Regression line of response variable chlorophyll b belongs on the y-axis against explanatory variable the mutagenic dose belongs on the x-axis.

Based on the performance of chlorophyll b against the mutagenic doses **(Figure 8)** the dependent and independent variables exhibited a significant positive correlation (r = 0.781) between both variables. This indicated that the changes of both variables are in the same direction, i. e., high concentrations of chlorophyll b are associated with the high doses of mutagenic agents inside the Figure and vice versa. However, the coefficient of determination (r) in this relationship was 0.781. This means that r^2= 0.6099, therefore 60.99 % of the variance in chlorophyll b concentration is due to the new genotypes induced by mutagenic agents. However, the remaining 39.01 % is due to other factors that were not taken in the analysis as environmental factors. The regression coefficient obtained between both variables was equal to 0.001. This means that chlorophyll b concentration was increased by 0.001 mg/g fresh weight (FW) with each additional unit of mutagenic agents. Therefore, the regression line represents the linear relationship between the concentration of chlorophyll b and mutagenic doses.

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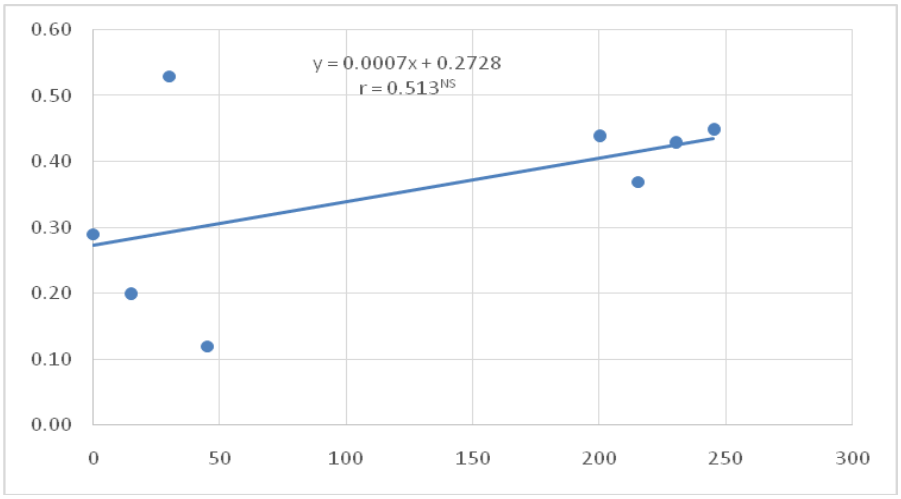

Figure 9. Regression line of response variable total chlorophyll belongs on the y-axis against explanatory variable the mutagenic doses belongs on the x-axis.

Total chlorophyll concentration in leaves exhibited an insignificant positive correlation (0.513) with mutagenic doses **(Figure 9)**. This positive value of correlation indicates that the changes of two variables were in the same direction, i.e., the high value of total chlorophyll concentration was associated with the high doses of mutagenic agents inside the Figure. Information regarding the coefficient of determination (r) in this relationship means that $r^2 = 0.263169$, therefore, 26.32 % of the variance in total chlorophyll concentration is due to new genotypes induced by mutagens. Meanwhile, the remaining 73.68 % is due to other factors that were not taken into this analysis. The regression coefficient of 0.0007 means that total chlorophyll concentration in leaves increases by 0.007 mg/g FW with each additional unit of mutagenic agents inside the Figure.

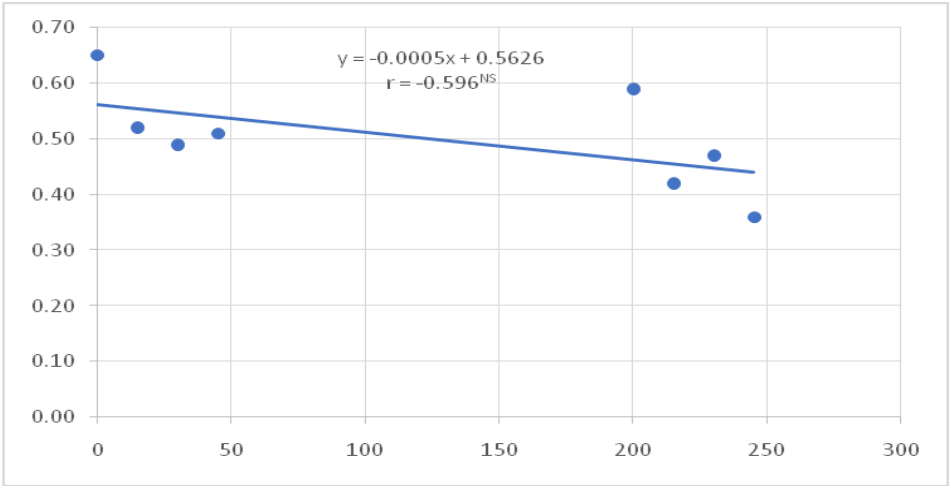

Figure 10. Regression line of response variable carotenoid in leaves belongs on the y-axis against explanatory variable the mutagenic dose belongs on the x-axis.

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Regarding to **Figure 10**, the correlation coefficient (r = 0.104) between carotenoid concentration in leaves with mutagenic doses showed an insignificant negative association between both variables. This means that the changes of two variables are not in the same direction, i.e., a high value in carotenoid concentration in leaves was associated with the lower doses of mutagenic agents and vice versa. In this relationship between both variables the regression coefficient was equal – 0.0002. This means that carotenoid concentration in leaves decreased by -0.0002 mg/g FW with each additional unit of mutagenic agents. However, the association between both variables r= = 0.104, therefore r^2 = 0.0108, this means that 1.08 % of the variance in carotenoid concentration is due to new genotypes induced by mutagens. Meanwhile, the remaining 98.92 % is due to other factors that were not included into this analysis. These results agreed with **Moore *et al.* (2013)**, who reported that if the correlation coefficient (r) between two variables is 0.30, then r^2 = 0.09, this means that approximately 9 % of the variance in the response variable can be due to the explanatory variable, the rest of the variance is attributed to the other factors that are not present in the regression equation.

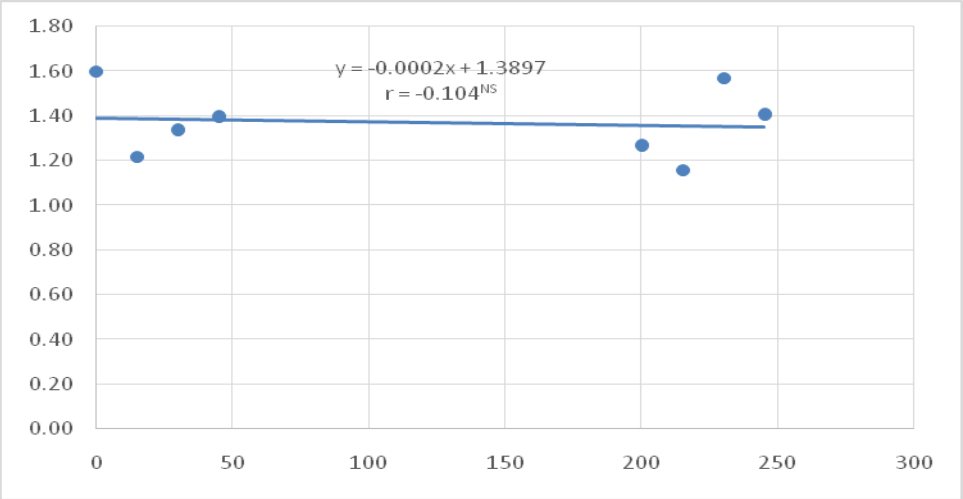

Figure 11. Regression line of response variable carotenoid concentration in pods belongs on the y-axis against explanatory variable the mutagenic doses belong on the x-axis.

According to **Figure 11,** the relationship between carotenoid concentration in pods with mutagenic doses exhibited a negative association (r = -0.596) between both variables. This means that the changes of two variables are not in the same direction, i.e., high values in the concentration of carotenoids in pods are associated with lower doses of mutagens. The regression coefficient of both variables was equal to -0.0005. This means that carotenoid concentration in pods was decreased by – 0.0005 mg/g FW with each additional unit of mutagenic agents.In interpreting the regression coefficient, one should recall which category of carotenoid concentration is represented by the higher doses of mutagens inside the Figure.

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The results obtained in this study agreed with **Tarkeshwar*et al*. (2020)**, who found that the number of spikelets per spike in wheat possesses a significant and positive correlation with plant height, spike length and biological yield per plant. Meanwhile, the same authors recorded negative associations between days to 50% flowering with peduncle length, number of grains per spike with days to maturity and harvest index with biological yield per plant. Meanwhile, **Asha *et al*. (2020)** recordeda positive and significant correlation in garden peas between the number of branches developedper plant with the number of pods per plant, whereas a negative significant association was found between the weight of ten pods and the number of seeds per pod. These traits can be used as a criterion during selection.

In conclusion, correlation shows the degree to which plant growth traits are associated with the doses of mutagenic agents. Genetic variability induced in the germplasm of garden peas by mutagenic agents used in this study describes their contribution towards the relative increase in plant growth traits which may be used as criteria for yield improvement in *Pisum sativum*. In addition, the regression line used in this study was to predict or explain the variation in plant growth traits based on the doses of mutagenic agents. It has already found a significant correlation between plant fresh weight, chlorophyll b with the mutagenic doses that induced new genotypes in garden pea. An insignificant positive correlation was obtained between plant dry weight, leaf area developed per plant, total chlorophyll concentration with the mutagenic doses. Therefore, a regression line describes how a plant's growth traits change as a mutagen dose changes. Regression analysis is a powerful and statistical procedure to predict the value of plant growth for a given value of mutagen doses. It enables geneticists to describe, predict and estimate the relationship between the interrelated variables.

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