Journal of Genetics, Genomics & Plant Breeding 7(4) 96-108 (October, 2023)
ISSN (Online): 2581-3293

RESEARCH ARTICLE

Genetic variability induced in chickpea by acridine orange

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Manuscript received:September 21,2023; Decision on manuscript:September 30,2023; Manuscript accepted:October 9, 2023

Abstract

This study aimed to increase genetic diversity in chickpea using acridine orange for improvement growth and yield traits using diverse genotypes. Six doses of acridine orange were used in this study in addition to the control against one genotype of chickpea. The seeds were planted in randomized complete block design with three replications. Treated populations were evaluated to study the extent of genetic variations induced in growth and yield parameters in M1 generation. Root growth was significantly increased above untreated plants in response to 80 ppm. The dose of 320 ppm generated the maximum concentration in total chlorophyll without any significant increase in carotenoids concentration at any of acridine doses. The dose of 20 ppm recorded significant increase in 100-seed weight above the control. The degree of homogeneity was differed within the same trait and from trait to another in the same genotype. Higher PCV and GCV were exhibited by plant fresh weight, plant dry weight, root length, carotenoids concentration, chlorophyll a, b, total, number of pods per plant and 100-seed weight. This study was highlight into genetic variability induced in chickpea and development of outstanding genotypes.

Keywords: Chickpea, acridine orange, diversity, growth, variability

Introduction

Induced biochemical mutations are the tools used by geneticists to study the function and organization of genes which are building blocks and controlthe plant growth and development. Mutations create raw materials for selection and genetic improvement of economical plants (Adamu *et al.,* 2004). Chickpea is self-pollinating crop legume, its genetic diversity is limited for its improvement techniques.Induced mutations seem an ideal technique for create of desirable genetic diversity (Mensahand Obadoni, 2007). In chickpea interspecific, intra-specific and intra-cultivar variation provides scope for breeding and selection for its improvement (Ashraf and McNeilly, 1992; Dudhe and Kumar, 2018). Induced mutations is an effective powerful tool because it is fast and cheap technique, caused heritable changes in the plant phenotype, as well as improved physiological, biochemical and quantitative traits as yield components via forming new genotypic varieties (Sangle and Lad, 2020).

Acridine orange is a chemical mutagen affecting on most metabolism processes in plant cell caused point mutations in chickpea, as well as proved to be very effective in the creation of mutants with high frequency (Okasha *et al.,* 2021). Acridine orange maybe effective in achieving early maturing mutants

of chickpea used to be increased pod yield (Animasaum *et al.,* 2014). The acridine dyes are flat, multiple ring molecules interact with DNA bases, as well as insert between them. This insert caused a "stretching" of the DNA duplex. Induced mutation plays a vital role in the creation of new crop genotypic varieties all over the world. Induced mutations in plant breeding technique led to generate 3.362 plant mutant genotypic varieties from 240 plant species among more than 75 countries all over the world (FAO/IAEA, 2020). Induced genetic variability is a major strategy in the improvement of all economical crops. Thus, insight into the magnitude of genetic diversity is importance to the plantbreeder for starting plant breeding technique. Exploiting of induced genetic diversity via mutation is a proven strategy in the improvement of all economically food crops. The essential prerequisite for any breeding programme is the available of genetic diversity in the gene pool of the economical crops. The narrow genetic base of chickpea as a self-pollinated crop referred to its cleistogamic flowers, considering it as the major constraint in plant breeding programme for crop improvement (Cubero, 1987).Therefore, breeding strategies in Chickpea needs to increase genetic diversity via incorporated new alleles through mutations induced to serve the objective of crop improvement. Thus, Yildirim *et al.,* (2013) advocated the importance of mutations induced in the gene pool of economical crops as an effective and efficient approaches to create and restore genetic diversity in chickpea. Plant breeding technique not only aims at improving crop productivity but also improved the quality traits through genetic variations induced in the genotypes. The development of superior varietal genotypes in plant resources known as mutation breeding (Kharkwal *et al.,* 2004). Therefore, this study aimed to investigate the impact of acridine orange as a chemical mutagenic agent on genetic variability induced in chickpea concerning morpho-physiological traits, as well as yield component traits, as well as determine the extent of variations observed referred to genetic factors.

Materials and methods

Chickpea genotype named Giza 195 was obtained from Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The selection of this genetic material based on its availability, as well as economic importance and tolerance to biotec stress. This investigation was executed in the Agri-Farm of Genetic Department, inside the Campus of Mansoura University during the Academic year of 2021/2022. Acridine orange penetrates acidic organelles in the cell as lysosomes because it is able to withstand in the low pH environments (Narjes *et al.,* 2019). Thus, it is able to penetrate the cell membranes of acidic organelles. Acridine orange was derived from the organic acridine, first isolated by boiling coal in Germany in the late of nineteenth century. Thus, acridine has antimicrobial agent useful for detecting drug-resistant bacteria (Stadler,1928). Acridine orange intercalate between the nitrogen bases in DNA molecule thereby caused distortion in the double helix of DNA, then DNA polymerase as a consequence recognizes this stretch as additional base and inserts its as extra opposite base in this stretched molecule leading to form frame shift mutations that altered the reading frame of DNA. They are more drastic in their effects because they are completely changes the gene message from the point of starting the addition or deletion leading to rearrangement of code bases in the DNA molecule (Oladosu *et al.,* 2016). The field experiment was done in a randomized complete block design with three replications. The healthy and viable seeds (moisture 11.0%) were treated with different concentrations of acridine orange at room temperature 25 ±4 for 12 hours of soak in beakers containing tap water.

Non treated seeds served as controls. All seeds were sown in the field trial to obtain M1 populations. The seeds sowing distance was 40 x 60 cm spacing. The M1 plants were harvested separately for measuring various morphological, physiological and quantitative traits. Data were collected and analyzed to assess the extent of genetic diversity induced. Observations of chlorophylls concentration were assessed at 50 days plant-old according to Torrecillas *et al.,* (1984).Although, the observations of various quantitative traits were measured at harvesting stage when the plants became to blooming. These including; plant height, number of primary branches, total number of pods per plant, seed yield (g/plant), plant dry weight (g) and hundred seed weight (g) (Amri-Tiliouine *et al.,* 2018). Control population was considered as standard. The seeds treated with acridine orange were thoroughly washed in running tap water for 15 minutes before sown in the field to remove the residual chemical from the seeds. The seeds were deposited in rows with three meters lengths, 40 cm spacing between plants and 60 cm width. The plants were thinned after complete emergence (which reached to 10 days after planting) to one plant per hill. The recommended practices by Ministry of Agriculture concerning chickpea production were applied at the proper time.

In this study growth and yield traits were assessment in M1 individual plants that may carries dominant mutant phenotype.

Homogeneity test

The level of homogeneity between treatments was measured according to Hallauer and Miranda (1988) using the following formula as follows:

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Population mean}}$$

The genetic variability parameters as genotypic variance (GV), phenotypic variance The results indicated that the dose of 80 ppm have a stimulatory effect on plant root length. (PV), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV), heritability (h2), genetic advance (GA), as well as genetic advance as percent of mean (GAM) for all traits studied in this work were assessment to understand the extent of which variations observed referred to genetic factors. These assessments were conducted according to Bammanakatti *et al.,* (2023). The data were subjected to the analysis of variance and the least significant difference (LSD) was used to compare between means if the differences between treatments where significant according to Duncan (1955).

Results and discussion

The results tabularized in Table 1appeared significant differences between the doses of acridine orange concerning plant fresh weight, plant dry weight, root length, plant height and number of primary branches per plant. These results are in harmony with Nithinkumar *et al.,* (2022), who obtained significant differences among the genotypes of bitter gourd for all studied traits indicating the presence of wide range of genetic variations in the genotypes. The root length was significantly increased above the control plants in response to 80 ppm. Plant fresh weight, plant dry weight, plant height and number of primary branches per plant are reduced with the corresponding increase in the doses of acridine orange, being maximum plant fresh weight (38.77 g) at 20 ppm and minimum (2.49 g) at 640 ppm, if compared with untreated plants (41.36 g).

The gradual decrease in plant height was recorded with the increase inacridine orange concentrations. The highest seedling height (96.33 cm) was observed in plants treated with 40 ppm, while the lowest (74.0 cm) was noted at 20 ppm, if compared with untreated plants (116.14 cm). The number of primary branches per plant was observed a gradual decrease with doses of acridine orange increased. Meanwhile, the dose of 640ppm was recorded an inhibitory effect for plant fresh weight, root

length and number of primary branches per plant. The inhibitory effect of acridine orange on the length of root seedling was evident from the decrease in the rate of mitotic cell cycle, as well as in cell elongation with increasing dose concentration.

Table 1: Mean values of phenotypic traits in M1 generation affected by different doses of acridine orange

Doses of acridine orange (ppm)	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Plant height (cm)	Number of primary branches/plant
00	41.36	350.42	55.35	116.14	100
20	38.77	176.15	46.95	074.00	063
40	09.07	190.60	50.00	096.33	055
80	09.94	064.79	57.75	086.83	077
160	06.52	202.40	46.95	094.96	043
320	14.06	257.30	42.00	088.18	039
640	02.49	253.40	24.90	092.11	026
F - test	**	**	**	**	**
LSD	2.43	41.98	1.24	27.96	2.63
	3.41	58.86	1.75	39.21	3.69

** = Significance at 0.01 probability level

The results obtained herein agreed with Gaul (1977), stated that the reduction in root and shoot length was attributed to the effects of mutagens on plant physiological system. The stimulatory effect on plant root length was observed at the lower dose of 80 ppm maybe due to the increase in cell cycle rate, as well as an activation of plant growth hormone, e.g. auxin and cell elongation (Zaka *et al.,* 2004). The primary physiological effect of plant growth hormone as auxin in plants is to stimulate cells elongation in shoot and root, as well as support the growth (Correa Aragunde *et al.,* 2004). The decrease in vegetative growth traits at the higher doses of acridine orange may be attributed to disturbances at cellular and physiological levels on meristematic tissues of the plant, leading alteration in physiological traits and change important components in plant cells, as well as decreasing mitotic activity. Thus, alkylating agents was reported to be the most effective and powerful mutagen tool typically produced only point mutations (Okagakiet al. 1991). Therefore, the decrease in branches number developed per plant due to treatment with acridine orange, probably due to slow cell division, suppressed initiated cells that developed new branches, slow cell elongation and lower synthesis of plant growth hormones or nucleic acids (Khursheed *et al.,* 2019). As shown in table 2, there were significant differences between the doses of acridine orange in the concentration of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. The combined concentration of chlorophyll a revealed a progressive increase with increasing doses of acridine orange, except for the doses of160 and 640ppm revealed a progressive decrease in this photosynthesis pigment. The maximum increase in this photosynthetic pigment (0.37 mg/g FW) was recorded by 40 ppm compared to the control (0.26 mg/g FW). The concentration of photosynthetic pigment chlorophyll b was ranged between 0.35 mg/g FW at 20 ppm to 0.85 mg/g FW at 320 ppm compared with untreated plants (0.51 mg/g FW).

This indicated that the maximum increase in chlorophyll b concentration was recorded by 320 ppm (0.85) followed by 160 ppm (0.83), 640ppm (0.80) and 80 ppm (0.64). The results did not indicate any significant increase in the concentration of chlorophyll a, total chlorophyll and carotenoid concentrations above the control. This may be due to exhibited production of free radicals at different doses of acridine orange which may be suppressed chlorophyll formation. The significant stable in carotenoid contents reflected its potent role in photo protection. Although, the results recorded significant increase in the concentration of chlorophyll b above the control plants at the higher doses of acridine orange (320,160 and 640 ppm). These results are in agreement with the previous findings of Rania *et al.,* (2020), who found augmented carotenoid contents in mutant lines of cowpea.

Table 2: Mean values of chlorophylls concentration in M1 leaves affected by acridine orange

Acridine orange dose (ppm)	Chlorophyll (a) mg/g FW			Chlorophyll (b) mg/g FW			Total chlorophyll mg/g FW			Carotenoid concentration mg/g FW
	60 days	12 days	Combined values	60 days	12 days	Combined values	60 days	12 days	Combined values	
00	0.20	0.35	0.26	0.41	0.62	0.51	0.61	0.96	0.78	0.25
20	0.12	0.47	0.29	0.22	0.49	0.35	0.34	0.96	0.65	0.16
40	0.11	0.63	0.37	0.21	1.03	0.62	0.32	1.66	0.99	0.14
80	0.18	0.54	0.36	0.33	0.95	0.64	0.48	1.49	0.98	0.25
160	0.15	0.19	0.17	0.30	1.36	0.83	0.45	1.58	1.02	0.21
320	0.22	0.39	0.31	0.39	1.31	0.85	0.62	1.70	1.16	0.27
640	0.22	0.12	0.17	0.37	1.23	0.80	0.59	1.35	0.97	0.25
F - test	Is	**	**	**	**	**	**	**	**	**
	0.03	0.11	0.21	0.05	0.11	0.19	0.08	0.18	0.50	0.035
	0.04	0.15	0.31	0.07	0.16	0.27	0.11	0.26	0.70	0.049

** = Significance at 0.01 probability level

The enhanced increase in the concentration of chlorophyllb at higher doses of acridine orange maybe attributed to their vital role in scavenging activity of free radicals during stress induced by a mutagenic agent (Fukuzawa *et al.,* 1998). The decrease in chlorophyll content has a negative impact on photosynthetic efficiency, since photosynthesis is dependent on light harvesting properties of chlorophylls (Gao *et al.,* 2004). On the other hand, carotenoids serve a protective mechanism against mutagenic agents (Rau *et al.,* 1991). This mechanism involves a photochemical change of singlet oxygen to triplet oxygen form via interaction with carotenoids which removing the dangerous oxygen radicals potentially produced in photo oxidative processes (Krinsky, 1979). The results tabularized in Table 3 revealed a progressive decrease in the mean values of yield and yield attributing traits with doses of acridine orange increased. This may be due to the mode of action of acridine induced physiological and biochemical disturbances and destruction of plant growth hormones as auxin and ascorbic acid content that may inhibit cell division and cell elongation (Fukuzawa *et al.,* 1998). The results also revealed that the number of pods developed per plant and pods weight per plant was decreased as the dose of acridine increased if compared with the control. This reflected the action of acridine orange for inducing sterile gametes that do not developed new pods because of genetic alteration during meiosis which generated unviable gametes. These results are in line with the findings of Sheeba et al.(2004), who found that sesame treated with ethyl methane sulfonate appear a linear decrease in seedling survival, delayed 50% flowering, seedling emergence and maturity with gamma doses increased.

In contrast, the dose of 20 ppm observed significant increase in 100-seed weight (59.38 g) if compared with that in the control (17.96 g). The number of pods developed per plant was ranged between 310 at 320 ppm to 114 at 80 ppm if compared with the control which recorded 326. Twenty ppm was the optimum dose of acridine orange stimulated growth regulators, enhanced enzymes activity as well as protein biosynthesis associated with 100-seed weight. This leading lower doses of acridine treatment could be employed for improvement the yield of chickpea genotypes and other related crops. The lowest decrease in the number of pods developed per plant (310) was recorded by320 ppm followed by 640 ppm and 40 ppm, if compared with the control which recorded 326. Meanwhile, the lowest decrease in the weight of pods developed per plant (131.75 g) was recorded by 320 ppm followed by 640 ppm (129.85 g) and 40 ppm (111.06 g). Whereas, the doses of 320 and 640 ppm were observed insignificantly increase in 100-seed weight above the control.

Table 3: Mean values of yield components in M1 generation affected by acridine orange

Doses of acridine orange (ppm)	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Plant height (cm)	Number of primary branches/plant
00	326	139.50	17.96	00	326
20	141	095.83	59.38	20	141
40	204	111.06	16.76	40	204
80	114	056.51	16.86	80	114
160	136	103.57	17.90	160	136
320	310	131.75	18.41	320	310
640	247	129.85	19.64	640	247
F - test	**	**	**	F - test	**
LSD	11.74	09.04	4.19	11.74	09.04
	16.46	12.67	5.88	16.46	12.67

** = Significance at 0.01 probability level

The weight of 100-seed is a reliable assessment of yielding ability in chickpea. The decrease in the number of pods was due to the decrease in the number of fertile flowers attributed to chromosomal disturbance producing sterile flowers as a result of the mode of action of mutagenic agent. The effect of mutagen on the auxin signals transduction caused increase in flower abscission and then reduced the number of pods developed per plant (Hayat et al. 2010). The decrease in pods number developed per plant if compared with control maybe attributed to late flowering and sterile gametes. Mutation breeding have resulted in two major outcomes: first improved varieties that are used directly for commercial production, second induced new genotypes with improved traits, as well as with better combining ability of economical traits. These traits could leading to be increased yield with early maturity, enhanced nutritional values and salt tolerance, etc. (Roychowdhury and Tah, 2013).

Degree of homogeneity was assessment depending upon coefficient of variance for phenotypic traits which used to estimate the magnitude of variation within doses of acridine orange (Table 4). The coefficient of variances for plant fresh weight was ranged from 0.003 at 20 ppm to 0.18at 320 ppm. The doses of 20 ppm (0.003) and 640 ppm (0.03)are reflected high homogeneity in plant fresh weight, where they are recorded their values lower to the check (0.04).The other doses including 40 (0.13), 80 (0.06), 160 (0.05) and 320 ppm (0.18) were produced high heterogeneity in this trait, while these doses recorded coefficient of variance (CV) higher than the check (0.04).

Table 4:Coefficient of variation for phenotypic traits in M1 generation affected by acridine orange

Doses of acridine orange (ppm)	Plant fresh weight (g)	Plant dry weight (g)	Root length (cm)	Plant height (cm)	Number of primary branches
00	0.040	0.050	0.03	0.06	0.04
20	0.003	0.003	0.02	0.09	0.06
40	0.130	0.010	0.07	0.10	0.04
80	0.060	0.010	0.02	0.02	0.02
160	0.050	0.030	0.03	0.05	0.02
320	0.180	0.080	0.06	0.03	0.04
640	0.030	0.070	0.02	0.10	0.04

Estimated coefficient of variance for plant dry weight was ranged between 0.003 (20 ppm) to 0.08 (320 ppm). The higher doses of acridine including 320 (0.08) and 640 ppm (0.07) could produce higher heterogeneous in plant dry weight, where they are gave higher variations within their plants than the check (0.05). For root length, the CV was ranged between 0.02 (20, 80 and 640 ppm)to 0.07 (40 ppm). The doses of 40 (0.07) and 320 ppm (0.06) could produce the highest heterogeneous in root length, where they are recorded the highest variations within their plants since they gave CV higher than that of the check genotype (0.03).

Estimated coefficient of variance for plant height was ranged between 0.02 to 0.10 compared with the check value (0.06). The coefficient of variation for number of primary branches per plant was ranged between 0.02 to 0.06 if compared with the check value (0.04). These results are in harmony with Berry and Rafique (1998), who selected different lines of tomato from F_2, F_3 and F_4 offspring and stated that the selected lines became higher homogeneous after F6 generation. In addition, Islam et al.(2011) selected segregated lines of tomato and evaluated them for high yield and virus resistant varieties. Meanwhile,Mahmoud and Khalil (2019)generated new local lines in tomato through selection from F2 population and the selected lines were observed enough homogeneous in all traits since they are exhibited lower CV values than that in the check genotype.

The results tabularized in Table 5 showed coefficient of variation for chlorophyll a was ranged between 0.02 to 0.22 if compared with the check genotype (0.08). The doses of 20 (0.22), 40 (0.11), 320 (0.10) and 640 ppm (0.12) could considered the highest heterogeneous in chlorophyll pigment concentration, since they are recorded higher variation values than those of the cheek (0.8).The coefficient of variation produced by the doses of 20 (0.05) and 640 ppm (0.03) were lower or close with that of the check genotype (0.05), indicating that the plants treated with these doses are high homogeneous in chlorophyll b, as well as they are more uniform in this trait than the plant streated with other doses of acridine. Meanwhile, the doses of 40 (0.07), 80 (0.06), 160 (0.08) and 320 ppm (0.08) are produced higher CV values than those of the check genotype (0.05), indicating high heterogeneity. The CV values of total chlorophyll were ranged between 0.03 to 0.10if compared with the check genotype (0.06). The doses of 20 (0.06), 160 (0.06) and 640 ppm (0.03) recorded CV values close or lower than those observed by the check genotype (0.06), indicating that the plants treated with these doses of acridine became highly homogeneous for total chlorophyll concentration, as well as they are more uniform in this trait than the plants treated with the other doses.

Table 5:Coefficient of variation for chlorophylls and carotenoids concentration in M1 generation affected by acridine orange

Doses of acridine orange (ppm)	Chlorophyll (a)	Chlorophyll (b)	Total chlorophyll	Carotenoids concentration
00	0.08	0.05	0.06	0.16
20	0.22	0.05	0.06	0.13
40	0.11	0.07	0.07	0.05
80	0.02	0.06	0.09	0.05
160	0.04	0.08	0.06	0.11
320	0.10	0.08	0.10	0.02
640	0.12	0.03	0.03	0.01

The doses of 40 (0.07), 80 (0.09) and 320 ppm (0.10) were observed higher CV values than that of the check genotype (0.06), indicating high heterogeneity, since they are reflected CV values higher than those of the check genotype. The plants treated with these doses of acridine orange were more varied in their contents of total chlorophyll than those treated with the other doses of check genotype.

Estimated coefficient of variance for carotenoids concentration was ranged between 0.01 (640 pm) to 0.16 (00 ppm), indicating high homogeneous in this trait, since all doses of acridine are reflected CV values close to or lower than those of the check (0.16).These results reflected that all doses of acridine orange are more uniform for their effects on carotenoids concentration leading their plants are more uniform in carotenoids concentration. For yield components, as shown in Table 6, the coefficient of variation for the number of pods developed per plant was ranged between 0.02 to 0.05.The doses of 20 (0.03), 40 (0.03), 160 (0.03), 320 (0.05) and 640 ppm (0.04) recorded higher values in CV than the chick genotype (0.02), indicating high heterogeneity in this trait. Meanwhile, the doses of 20 (0.04), 40 (0.07), 160 (0.08) and 320 ppm (0.06) recorded higher CV values than those of the check genotype (0.02), indicating high heterogeneity for the weight of pods developed per plant. This observations reflected that the plants treated with these doses of acridine orange were differed in their products concerning the weight of pods developed per plant due to variations in their fertility of flowers, because of chromosomal abnormalities and point mutations generated by acridine orange in the sexual organs of checkpea. Variation coefficient of 100-seedweight was ranged between 0.01 (00, 80 ppm) to 0.21 (20 ppm) if compared with the check genotype (0.01).The dose of 80 ppm (0.01) is the only dose recorded CV value close to the check genotype (0.01), which could be considered homogeneous for this trait, as well as their plants are more uniform than other streated with the other doses of acridine orange.

Table 6: Coefficient of variation for yield components in M1 generation affected by acridine orange

Doses of acridine orange (ppm)	Number of pods/plant	Weight of pods per plant (g)	100-seed weight (g)
00	0.02	0.02	0.01
20	0.03	0.04	0.21
40	0.03	0.07	0.09
80	0.02	0.01	0.01
160	0.03	0.08	0.03
320	0.05	0.06	0.04
640	0.04	0.02	0.07

These results agreed with El-Morsy *et al.*, (2021), who selected 16 new lines of tomato from sex F_2 generations based upon high homogeneity obtained from CV values for plant height, number of leaves per plant, shape index, hardness, vitamin C and TSS%. Ahmed et al. (2017) decided that the estimated coefficient of variance which reflected degree of homogeneity was differed among genotypes for the same trait, as well as from trait to another within the same genotype. Thus, heterogeneous obtained in different economical traits assessed in this study has referred to genetic variability induced by acridine. These genetic variations has contributed to modern plant breeding which played a major role all over the world in the development of superior plant varieties or genotypes (Kharkwal and Shu, 2009).

The results in table 7 and 8 pertaining to genetic parameters revealed that the estimates of GV were higher than the estimates of corresponding EV for the following traits; plant fresh weight, plant dryweight, root length, carotenoids concentration, number of branches, chlorophyll a, b, total chlorophyll, number of pods per plant, weight of pods per plant and 100-seed weight. Meanwhile, plant height showed high EV exceed the estimate of corresponding GV. The higher estimates of GCV (>20%) were recorded for plant fresh weight (143.2%), plant dry weight (224.7 %), root length (34.5 %), plant height (35.1 %), number of branches (28.9 %), chlorophyll a, number of pods per plant, weight of pods per plant and 100-seed weight. The higher estimates of ECV than the corresponding estimates of GCV were recorded for plant height (35.9 %) and chlorophyll b (11.4 %). The PCV values were ranged from 4.3 % (carotenoids concentration) to 232.9 % (plant dry weight). The higher estimates in PCV exceeds 50% were recorded for the following traits; plant fresh weight (143.8 %),plant dry weight (232.9 %), plant height (70.9 %), number of pods developed per plant (223.8 %), weight of pods per plant (103.4 %) and 100-seed weight (130.5 %). These results agreed with Mawuli *et al.*, (2022), who found that the phenotypic coefficient of variation was greater than the corresponding values of genotypic coefficient of variation for all traits studied in garden eggs indicating some level of environmental effects on these traits. Whereas, the lower estimates in genetic advance as a percentage of mean were recorded for the number of branches per plant (18.4%) followed by plant height (31.8%),carotenoids concentration (39.8 %), total chlorophyll(42.5 %) and root length (46.8 %).

Table 7: Assessment of genetic parameters concerning growth characteristics and yield component traits in M1 generation affected by acridine orange

Trait	Genetic variance	Phenotypic variance	Environmental variance	Heriatability (%)
Plant fresh weight	250.6	252.5	1.9	99.3
Plant dry weight	7551.0	8107.8	556.8	93.1
Root length	12.9	13.4	0.5	96.3
Plant height	79.8	326.9	247. 0	24.4
Carotenoids concentration	0.0	0.0	0.0	85.7
Number of branches per plant	21.9	43.2	21.3	50.8
Chlorophyll (a)	0.0	0.0	0.0	93.9
Chlorophyll (b)	0.1	0.1	0.0	96.6
Total chlorophyll	0.0	0.1	0.0	89.4
Number of pods per plant	7366.4	7410.0	43.6	99.7
Weight of pods per plant (g)	795.8	821.6	25.8	96.9
100-seed weight	244.6	250.2	5.6	98.9

Table 8: Assessment of genetic parameters concerning growth characteristics and yield component traits in M1 generation affected by acridine orange

Trait	GCV (%)	PCV (%)	Environment coefficient of variation (%)	Expected genetic advance	Genetic advance as percent of mean (%)
Plant fresh weight (g)	143.2	143.8	0. 6	32.4	185.5
Plant dry weight (g)	224.7	232.9	8.1	172.5	80.8
Root length (cm)	34.5	35.2	0.7	7.2	46.8
Plant height (cm)	35.1	70.9	35.9	29.4	31.8
Carotenoids concentration	3.9	4.3	0.3	0.1	39.8
Number of branches per plant	28.9	40.6	11.7	6.9	18.4
Chlorophyll (a)	28.1	28.9	0.9	0.3	89.7
Chlorophyll (b)	1.6	12.9	11.4	0.7	67.9
Total chlorophyll	9.8	10.3	0.5	0.6	42.5
Number of pods per plant	223.1	223. 8	0.7	177.3	83.9
Weight of pods per plant (g)	101.8	103.4	1.6	56.7	51.6
100-seed weight (g)	129.0	130.5	1.4	31.9	133.9

Deshmukh *et al.*, (1986) recorded PCV and GCV values exceeds 20% categorized as high, whereas values between 10-20% regarded as medium, as well as values below10% regarded as low. If the heritability value is very high, then this considered 80% or more, selection could be easy for these traits due to small contribution of the environmental factors on the phenotypic performance (Singh 2001). In addition, Johnson et al. (1955) categorized genetic advance as percent of mean to three classes as follows, low (0-10%), medium (10-20%) and high (above 20%). Therefore, traits exhibited very high heritability values indicating the minimum effect of environmental factors on the phenotype of traits leading selection would be effective (Singh, 2001). In contrast, traits exhibited very high heritability values indicating the minimum effect of environmental factors on the expression of traits, because they are involvement of additive gene action in their inheritance leading selection would be effective in their improvement. These traits including plant fresh weight, plant dry weight, root length, carotenoids concentration, chlorophyll a, b and total, number of pods per plant, weight of pods per plant and 100-seed weight. In addition, Johnson *et al.*, 1955) decided that heritability values coupled with genetic advance is usually more helpful than heritability value alone in selecting the superior genotypes that predicting the resultant effect. The results obtained in this study agreed with Bammanakatti *et al.*, (2023), who stated that traits observed high PCV and GCV indicated a wide variations and they are governed by additive gene action enables plant breeder to design effective selection technique. Amri-Tiliouine *et al.*, (2018) in chickpea, recorded high estimates in genetic parameters for the number of seeds per plant and seed yielding.

Therefore, selection based upon phenotypic performance of the genotypes will lead to increase the mean performance of the selected offspring. Through this study the objective to study genetic variability induced in chickpea and development of useful genotypes for the future chickpea breeding gets fulfilled.

Hence, in conclusion, seedling growth traits and yield components were mutagenic dosage dependent upon the concentration of acridine orange used. Mutation breeding is a major component in developing novel genotypes in relatively short time. Chickpea genotype responded differently to different doses of acridine orange. High doses caused considerable decrease upon the traits studied. The success achieved in chickpea mutation breeding indicates that it is still an important technique in the development of new genotypes in chickpea expressed superior phenotypes in different traits. Yield in chickpea is a sum total of contribution by many simply inherited traits, therefore direct selection for yield must be correlated with many contributing traits. These traits were generally correlated each other, as well as with the yield performance.

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