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GERMINATION SCENARIO AND GROWTH ANALYSIS FOR IRRADIATED COWPEA

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Abstract

Gamma - ray technique, as physical mutagens method, substitute classical breeding methods, for development a new varieties, to face of population explosion threats, and limited of agricultural production elements (water + agricultural area). Cowpea seeds *cv.* Kafr El– Shiekh, were exposed by six γ- doses “γ_d”; 0, 50,100, 150, 250 and 350 Gray (Gy). Pretreated seeds were germinated to predicted lethal dose (LD_{50}) and observed germination scenarios. Thereafter, exposed seeds by 0, 50, 100 and 150Gy., were grown till maturity at open field to evaluate growth characterizes *i.e.*, leaf area index “LAI”, specific leaf weight “SLW”, net assimilation rate “NAR”, and relative growth rate “RGR”. As well as, nodules number per plant “NNP”, nodules dry mass per plant “NDM”, and seed yield “Sy”, using complete randomized block design. Results affirmed that LD_{50} was $\approx$ 200 Gy. Gamma ray has a bio-positive significant effect on germination indices. Where, max. germination percentage “GP” (92%), mean germination time “MGT”(1.87day), highest mean daily germination “MDG” (30.67z% day^{1}), and germination index “GI” (380) were obtained by exposed seeds with 150Gy. Also, the development of the growth parameters LAI, SLW, NAR, RGR, affected significantly ($P \leq 0.05$) by γ_d from plant establishment to maturity. NNP had direct relations with γ_d, at different plant age. Then, the increase in “Sy” was more pronounced and significantly affect with pre-sowing seeds irradiation, compared with control.

Key words: Cowpea seeds, gamma irradiation, germination indices, growth characterizes nodules parameters, and yield.

Introduction

Cowpea, *Vigna unguiculata* L. Walp. (Family: Leguminosae), is very important to prefatory protein gap in food security. It has been referred as poor man’s meat, cause it coincidentally equals to the protein in meat (18-40%). So, it consider a cheap alternative to increase Egyptian citizen allowance from protein. Where, they are consumed about 32kg year^{-1}., from animal protein (11.4, 10.4, and 10.2 kg.year^{-1}., from meat, poultry and fish respectively) represented about 26.95% from world consumption (118.7 kg.year^{-1}) (Ugorji *et al*., 2012 and EAS, 2016). It may consumed as green leaves, pod, and peas, dry grains, and it’s excellent animal feed as straw, hay, silage, and pasture. It’s a deep rooted crop, has ability to improve sandy soil fertility, through nitrogen fixation and as a cover crop to suppress nematode in tomato production system (Abayomi *et al.*, 2008, and Ibrahim *et al*., 2010). It can fix about 100.8 kg fed^{-1}., of atmospheric nitrogen and make available about 25.2-29.2 kg fed^{-1}., N, for next crops grown in rotation with it (Aikins and Afuakwa, 2008). Also, it’s one of the drought resistant crops and withstand salinity (Trivedi *et al*., 2015). As well as, it reduced weeds, pest, and diseases (Chemining wa and Vessey, 2006). Lastly, It’s good source of carbohydrate, minerals (potassium, phosphorus, calcium, iron, copper, zinc and boron), vitamins and carotene (Amjad *et al*., 2006).

Mutation induction has become an established tool in plant breeding to improve cultivars in certain specific traits (higher yield, and better grain quality). It can be achieved by using gamma ray technique, as physical mutagens method, substitute classical breeding methods. Because it’s relatively safe, non-toxic, and cost-effective (Jain, 2010). Gamma ray is an electromagnetic ionizing radiation, having energy level ranging from 10 kV., to several hundred kV., (Kovacs and Keresztes, 2002), with short wavelength which has the tendency to release energy as its passes through a tissue. The principal effect of ionizing radiation is the ionization of water molecule "H_2O^{++} (hydroxyl radicals), producing H^- and ^-OH radicals. These ionizations are induced all along the radiation path, in biological tissues, leading to produce secondary free radicals through chain reactions as a result of H^+ and e^-_{aq} becoming trapped (Esnault *et al.*, 2010). These radical react with cellular components, especially DNA to cause mutations. (Olasupo *et al*., 2016). A large dose scale has been tried to establish possible changes, lethal dose (LD_{50}), and appropriate mutation dose through germination and survival percentages (Mba, 2013). Where, low doses of γ-irradiation have been used for mutant isolation in conventional plant breeding, increasing growth and yield of plants (Mudibu *et al*., 2011). However, high doses disturbs the protein synthesis, hormone balance, enzyme activity, and cell growth comes to a halt (Hameed *et al*., 2008).

Reducing germination time and accelerate the growth are the beneficial effects of γ- radiation in improving the crop (Akshatha and Chandrashekar, 2018). However, Ulukapi and Ozmen 2018., resulted that gamma application (0 till 500Gy), have no significant influence on common bean germination percentages, but only significant decreases in surviving plants number, were notice with dose increase. On the other hand, not only germination percentage "GP" (%) of seeds are used as parameters when the effectiveness of gamma rays is examined, but also, the speed, time, rate, homogeneity, synchrony and distribution of this germination are aspects, used to judge on agronomic relevance and informing the dynamics of the germination process (Ranal and De Santana, 2006). Moreover, it has impacts on diverse cultural operations *i.e.*, fertilizing, harvesting and field maturity of crops (Kader and Jutzi 2001).

Growth characteristics *i.e.,* Leaf area index "LAI", specific leaf weight "SLW" (mg cm^{-2}), net assimilation rate "NAR" (g $cm^{-2}d^{-1}$), and relative growth rate "RGR" (g $g^{-1}d^{-1}$), provided an understanding of the growth and crop development throughout the period of growth. LAI, is the primary factor that determines the rate of dry matter production in a closed stand, and describes the size of the assimilatory apparatus of the plant stand. Where, it used as indices for improving seed yield of lentil (Haghnazari *et al.,* 2005). Specific leaf weight "SLW", is reciprocal to specific leaf area "SLA". So leaves with higher SLW and/or lower SLA are thicker. Thicker leaves (≈ high SLW) have been used as a tool to screen cultivars for productivity because there are related with high yielding capacities crop cultivars (White and Montes, 2005). Moreover, SLW can be used as an indirect measure of several basic leaf processes, *i.e.*, photosynthetic capacity and growth rate (Negative relationship) (Dong *et al*., 2011). NAR is effective indices in increasing yield, defended as the rate of increase in plant mass per unit leaf area. Where, Katsura *et al*., 2007, reported that rice grain yield can be increased by selection on the basis of physiological growth indices like NAR. The important of high RGR could be a high plant mass after a certain period of growth, and also facilitate rapid completion of life cycle of plant Due to high RGR, a plant will quickly increase in size and the able to occupy a large space, both under and over the ground (Ahmadi *et al*., 2014).

It is on this premise that this paper seeks to achieve two goals, through laboratory and field experiments. Lab. exp. aims to observing the effects of different doses of gamma ray irradiation on germination scenario and determined lethal dose of cowpea cultivar under studied. Meanwhile, field exp. undertaken to investigate safety doses effecting on growth traits *i.e.*, LAI, SLW, NAR, and RGR., nodules parameter, and seed yield.

Materials and methods

A homogenous lot of certified cowpea (*Vigna unguiculata* L.) dray seeds *cv.* Kafr El–Shiekh, produced by private company (Mecca Trade), divided into 6 groups of 5 kg., each, and treated with ^{60}Co (Cobalt 60) gamma rays, in ambient conditions of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Dry seeds (moisture content ≈ 12 to 14%), were sealed in low density polyethylene (LDPE) pouches, and exposed to different doses of γ-rays; 0 (control), 50, 100, 150, 250 and 350 Gray (Gy), by Egypt's Mega Gamma^{-1}, of the type J-6500 supplied by the Atomic Energy of Canada limited. Ferrous ammonium sulphate (Fricke) dosimeters used to determine the absorbed dose in the treatments. High Frequency Dosimeters System, model 2131, version 2.5, Sensolab Ltd. used as instrument to calibrate absorbed dose.

Five replicates of 100 seeds, from exposed seeds (0, 150, 250 and 350 Gy) were sowed on moistened blotting paper, and then germinated under lab. conditions. Germinated seeds were counts daily for 5 days. Seeds were considered germinated when radicals reached a length more than 2mm. Bioassay experiments were conducted to guess lethal dose (LD_{50}) through germination percentage "GP" (%), and seedling survival percentage "SSP" (%). Other germination indices i.e., mean germination time "MGT" (day), germination rate index

"GRI" (% day^{-1}), germination index "GI", mean daily germination "MDG" (% day^{-1}), and coefficient of germination of velocity "CVG" (%), "were calculated also as hereinafter equations.

$$\text{GP} = \frac{N_t}{N}\times 100 \text{ ....(Kader, 2005)}$$

$$\text{SSP} = \frac{N_S}{N}\times 100 \text{ ....(Olasupo } et\ al.\text{, 2016)}$$

$$\text{MGT} = \frac{(N_1\times d_1)+(N_2\times d_2)+...+(N_n\times d_n)}{N_T}$$

.... (Kader, 2005)

$$\text{GRI} = \frac{G_1}{d_1}+\frac{G_2}{d_2}+\frac{G_3}{d_3}+...........\frac{G_n}{d_n} \text{ .... (Kader, 2005)}$$

$$\text{GI} = (5\times n_1) + (4\times n_2) + \dots + (1\times n_5) \text{ .. (Kader, 2005)}$$

$$\text{MDG} = \frac{GP}{D} \text{ .... (Gairola } et\ al.\text{, 2011)}$$

$$\text{CVG} = \left(\frac{N_1+N_2+N_3+..........Nn}{(1\times N_1)+(2\times N_2)+(3\times N_3)+.........(n\times N_n)+}\right)$$

......(Ranal and De Santana., 2006)

Where: N_T = No. of germinated seeds in each treatment; N = number of seeds used in the bioassay (=100); N_S = Seedlings survival in each treatment; N_1 to N_n= seeds No. that germinated in day 1, 2, to n; d = Number of days; n = No of counts; G_1, to G_n. = Germination percentage at that germinated in day 1, 2.. .....; 5, 4, ….. and 1 = are weights given to the number of germination seeds on the first, second and subsequent days, respectively; D = Total No. of days (= 5 day)

Therefore, open field exp. was carried out during Nile season (18/6 to 28/9/2017), at private farm, El-Behiara governorate, Egypt., at $24^{0}07'$ N lat., $30^{0}44'$E long., and at an alt. of 12m above sea level. Experimental soil was classified as sand in texture (5.40% clay, 93.25% sand, and 1.25 silt), with bulk density "ρ" ≈ 1.62 g cm^{-2}., pH (1: 25) ≈ 8.02., electric conductivity "Ec" ≈ 0.25 ppm., total N ≈ 3.22 ppm., and organic matter "OM" ≈ 0.100%. Soil physical and chemical analyses were analyzed according to Tan (1996), meanwhile, textural class name was ascertained from the textural triangle as described by Piper (1950).

Exposed (50, 100 and 150 Gy) and control seeds were planted randomly in four blocks, with four replicates arranged in complete randomized block design with total area 1369m^2 ≈ one third feddan (feddan or fed.: Egyptian area unit = 4200m^2). Each experiment unit was (8 × 8m) include 16 ridges. A distance of 1m., was left between experiment unit as buffer zone. Seeds inoculated by *Bradyrhizobium sp. Vignae*, with rate of 150g., inoculate per 30 kg., seed, prior planting. Herati method, (Herati = Egyptian expression for planting on 75%., from water field capacity), used to planting, with a rate of 20 kg fed^{-1}. Three seeds per hole, planting distance of 10 cm., within rows. All agricultural practices *i.e.,* fertilizing, thinning, hoeing, weeding and sprays against insects, pests and diseases were followed throughout the growing seasons as recommended by Ministry of Agriculture.

During the growing season, twenty randomly plant samples were uprooted, five times at 21, 42, 63 and 84 days after planting (DAP), and at harvest day from each treatments. Whole plant mass were recorded by digital electrical balance with an accuracy of 0.001g. Leaf area "L_a" (cm^2) was estimating by gravimetric method (Jonckheere *et al.*, 2004). Leaf area, and plant mass were established in following formula to obtain; leaf area index "LAI", specific leaf weight "SLW" (mg cm^{-2}), net assimilation rate "NAR" (g cm^{-2}d^{-1}), and relative growth rate "RGR" (g g^{-1} d^{-1}).

$$\text{LAI} = \frac{L}{P} \text{ .... (Addo-Quaye } et\ al.\text{, 2011)}$$

$$\text{SLW} = \frac{L_m}{P_a} \text{ .... (Amanullah, 2015)}$$

$$\text{NAR} = \frac{(W_2-W_1)}{(t_2-t_1)}\times\frac{(In\,A_2-In\,A_1)}{(A_2-A_1)}$$

.... (Addo-Quaye *et al.*, 2011)

$$\text{RGR} = \frac{InW_2-InW_1}{t_2-t_1} \text{ .... (Anjum and Bajwa, 2005)}$$

Where: L, is one half of the total leaf area (cm^2); P, net sample area (≈ 500cm^2); L_m, leaf mass (mg); L_a, leaf area (cm^2); W_1 and W_2., are plant dray mass (g) at times t_1 and t_2 (e.g. in days) respectively; ln, is natural logarithm; A_1 and A_2 are the leaves area at times t_1 and t_2., respectively.

Also, twenty plants samples were uprooted carefully at 30, 45 and 60 DAP. Each plant root was washed and carefully rinsed with water. The nodules were harvested, counted and recorded to determine No. of nodules per plant "NNP" (^{1}.plant^{-1}), and nodules dry mass per plant "NDM" (mg plant^{-1}). All samples were dried in electric oven at 70˚C., for 4 days., until constant weight. At harvest day "Hd", (100 DAP), as 95% from matured pods on the vine were dry. Plants were uprooted, sun dried for one week, and threshed. Seeds of each block were weighted and mean seed yield in Mg fed^{-1}., were calculated. Data were subjected to analysis of variance (ANOVA) according to the procedure of Snedecor and Cochran (1980). The mean values were compared at 5% levels of significant difference (LSD- test).

Results

Figs 1 to 5, show germination scenarios for exposed seeds by 0, 150, 250, and 350Gy. Gamma - rays has a stimulatory significant effect on germination percentage "GP" (%) till specific dose, (Akshatha *et al*., 2018), and sharply decreased thereafter with increasing gamma doses "γ_d", (Guar *et al*., 2003 and Toker *et al*., 2005). This behavior were noticeable clearly in Fig. 1, where, max. GP (92%), was recorded at 150 Gy., followed by 81%., at untreated seeds (control). Then, a steep decrease to 48 and 32%., at 250 and 350Gy., these are in agreement with this obtained by Dhanavel *et al*., 2012 and Mohammed *et al*., 2018, but contrary with mention by Badr *et al*., 2014. With observing Fig. 2, there were sudden decrease in survival seedlings "SSP" (%) and retarded seed *i.e.*, delayed emergence, and distortion seeds (seeds did not succeed in continuing radicals growth and plumule emergence), at dose more 150Gy. Therefore, by representing the relation between survival seedling percentage "SSP" and "γ_d", show the lethal dose "LD_{50}" at ≈200 Gy., predicted through herein after regression equation,

$y = 3\times10^{-5}\ g_d^{3} - 0.0143\ g_d^{2} + 1.5643\ g_d + 100$., with $R^2 = 1$., Fig. 3. Whilst, LD_{50}, varies by plant varieties and within species (Ulukapi and Ozmaen 2018).

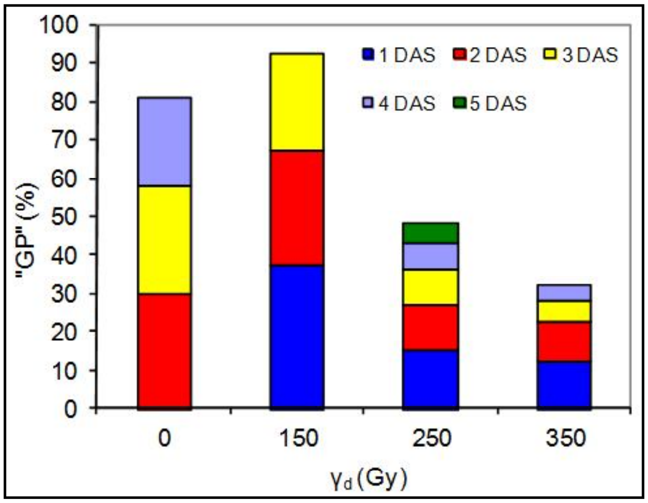

Fig. 1: Germinated seeds percentage Vs. gamma doses "γ_d" during five days after sown.

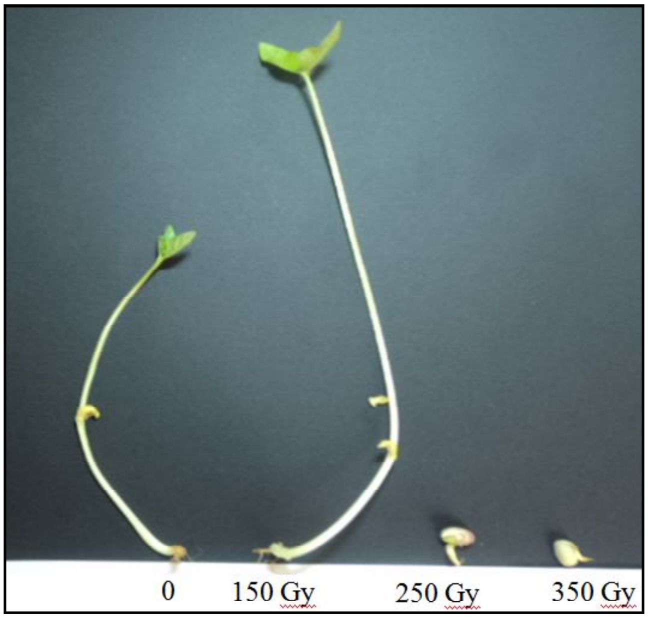

Fig. 2: Morphology appearance of cowpea seedling five days after sown.

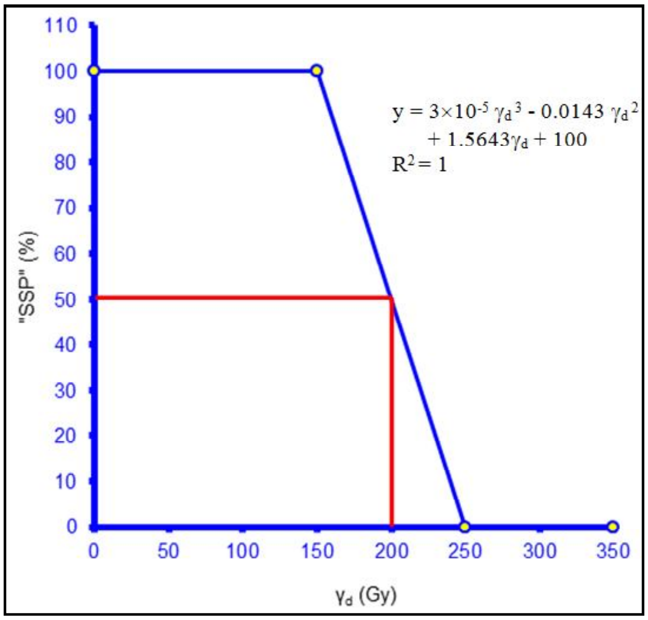

Fig. 3: Survival seedling percentage "SSP" in response to gamma doses "c".

Fig. 4 and 5., showed that bio-positive effects was found by treated seeds with 150Gy., and revealed the same declining pattern by increasing γ_d more than LD_{50}. However, the correlations of germination parameters could be summarized as following equations;

$GP = 10^{-5}\ \gamma_d^{3} - 0.006\ \gamma_d^{2} + 0.7513\ \gamma_d + 81$., with $R^2 = 1$.,

$MGT = -3\times10^{-7}\ \gamma_d^{3} + 0.0002\ \gamma_d^{2} - 0.0259\ \gamma_d + 2.9136$., with $R^2 = 1$.,

$CVG = 6\ ´10^{-6}\ \gamma_d^{3} - 0.0034\times10^{-5}\ \gamma_d^{2} + 0.5086\times10^{-5}\ \gamma_d + 34.322$., with $R^2 = 1$.,

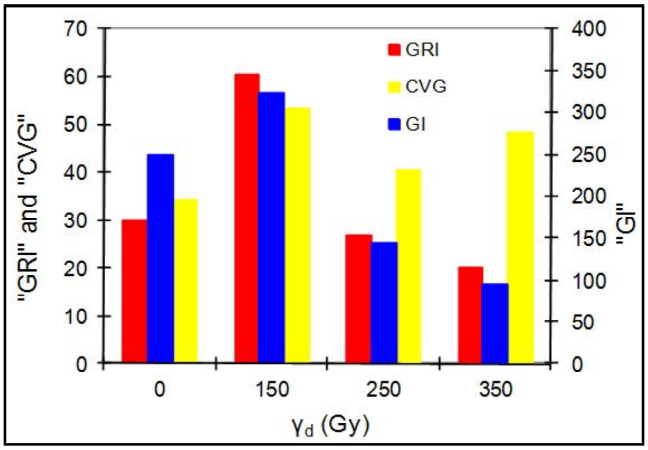

Fig. 4: "GRI", "CVG" and "GI" Vs. gamma doses "γ_d" during five days after sown.

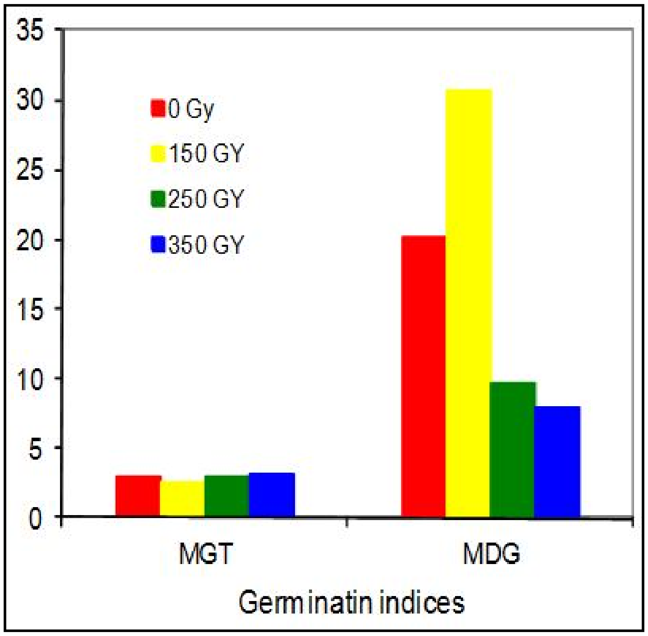

Fig. 5: "MGT" and "MDG" at different gamma doses "γ_d" during five days after sown.

GRI or SG = $12.388\ e^{0.0147GP}$., with $R^2 = 0.7726$.,

GI = $73.426\ e^{0.0168\ GP}$., with $R^2 = 0.9583$.,

MDG = $3.6145e^{0.0223\ GP}$., with $R^2 = 0.9752$. (Data not shown)

Whereas, it's not logical to discuss the effect of doses more than LD_{50}. (≤ 200 Gy) on germination parameters. Of late results, GP is unsatisfactory indicator to evaluate irradiation effective. Where, MGT was 1.87 day., faster than control 2.91day., Meanwhile, both MDG and GI were increased from 20.25 to 30.67 % day^{-1}., and from 250 to 380. As well as, GRI was increased about two-fold. Germination rapidity » CVG was rapidly (from 34.32 to 53.49). Similar results were demonstrated by Selvaraju and Raja, 2001.

Generally, it was remarkable to note however, that from plant establishment to maturity the development of the growth parameters LAI, SLW, NAR, RGR, followed similar patterns, and affected significantly by γ_d.

Leaf area index "LAI" has direct proportion with plant age till 63 DAP (Day after planting), thereafter the relation has negative proportion till harvesting (Fig. 6). Control seeds at 21 DAP., ranked last in terms of LAI (0.083), meanwhile, Plants of seeds irradiation "PSI" with 150 Gy., has the heights LAI (0.266) at 63 DAP. Initially, LAI curve of control was lower than exposing seeds. LAI, value for control treatment was improve slightly from 21 to 84 DAP (56.49%) with augmenting percentage about 16.67%., as well as, its value decline slightly about 5.07% from 84 DAP, to harvest day (Hd). LAI were increase sharply during 42day., (from 21 to 63 DAP) about 58.63, 59.22 and 3957%., for PSI, with 50, 100, and 150Gy., respectively. Then, it decline slightly, about 10.22, 9.66, and 13.04% during last 37day., (from 63 to Hd), with same previous arrangement, respectively. The highest increasing rate of LAI (35.03%) followed by (31.56%) were obtained within 21 to 42 DAP., at PSI 50Gy., and control. Although, there were significant differences in the LAI with exposing different doses. It's exciting to note that, Akshatha *et al*., 2018., observed that by increasing γ_d, till 200Gy., both of leaf area and length, terminal leaflet length and width, and leaves number were increase by increasing gamma dose till 200Gy. Contrary, Akshatha and Chandrashekar, 2018, resulted that irradiation did not show any impact on leaf number, as well as, As are *et al*., 2017., resulted that leaf length, width and numbers were decreasing by increase doses from 400 to 1000Gy on okra plant.

Specific leaf weight "SLW" (mg cm^{-2}) one of the easiest components of growth to measure. The relation of SLW Vs. γ_d is presented in Fig. 7. PSI with 150Gy., at 84 DAP, ranked first in terms of SLW (83.51 mg cm^{-2}), and untreated plants had the lowest SLW (21.14 mg cm^{-2}) at 21 DAP. Also, SLW curve of untreated seeds was initially lower than PSI. Leaves thickness (≤ SLW) were increase sharply (about two – fold) from 42 to 84 DAP, then, ascend steadily and sharply too (about one third) from 84 DAP to Hd. In general, there was a direct proportion with exponential correlation between SLW and plant age from 21 to 84day (Data not show). Afterward, the relations became negative from 84 DAP to Hd. Data show that γ_d, significantly affected on "SLW" at all DAP., but the difference did not approach to significant level for "PSI" with 50 to 100Gy.

Net assimilation rate NAR for treatments under studied ranged between 0.098 to 0.951 g cm^{-2} d^{-1}. Inherent differences in NAR associated negatively with

SLA subsequently associated positively with SLW (Villar *et al.*, 1998). PSI with 150 Gy., recorded the largest NAR followed by 100, 50 and 0 Gy. Highest and lowest rate of NAR were 93.85 and 11.79%., were obtained at untreated seeds and PSI with 150Gy., within plant age from second to third intervals (21-42 to 42-63), and from third to fourth intervals (42-63 to 63-84 DAP). Also, NAR had a positive proportion with sampling intervals from first till third intervals (0-21 to 42-63 DAP). Meanwhile, these proportions were $NAR \approx 0.038\ e^{0.8558\ (DAP)} \approx 0.0722e^{0.741(DAP)} \approx 0.1461e^{\ 0.5681(DAP)} \approx 0.2242e^{\ 0.4675(DAP)}$., for control and PSI with 50, 100 and 150Gy., respectively. (Fig. 8).

Fig. 9. revealed that relative growth rates “RGR” for control and PSI ranged between 15.26 and 75.48 mg $g^{-1}d^{-1}$. PSI with 150Gy., displayed as faster RGR (35.48 mg $g^{-1}d^{-1}$) at sampling intervals 0-21 DAP., than the others. While, RGR has a positive proportion with sampling intervals till fourth intervals, where, RGR increased as plant size increased. Where RGR increased within sampling intervals from first till fourth intervals (0-21 to 63-84 DAP) about 136.89, 141.63, 104.68, and 112.73%. Thereafter, decline slightly from fourth to fifth intervals (63-84 to 84-Hd), about 5.25, 13.2, 9.75, and 7.83%., for PSI with 0. 50, 100 and 150Gy., respectively. The rate of RGR growing slowly negatively from first to second and from second to third intervals (0-21 to 21-42 and 21-42 to 42-63 DAP) about 32.24 and 29.13%, 37.42 and 30.33, 25.94 and 18.39%, and 31.21 and 16.61%., for control and PSI with control, 50, 100 and 150Gy., respectively. PSI with 150Gy., scored the highest rate of RGR (39.03%) within third to forth intervals (45-63 to 63-84 DAP), also the lowest rate (5.25%) was obtained with control treatment within fourth and fifth interval samples (63-84

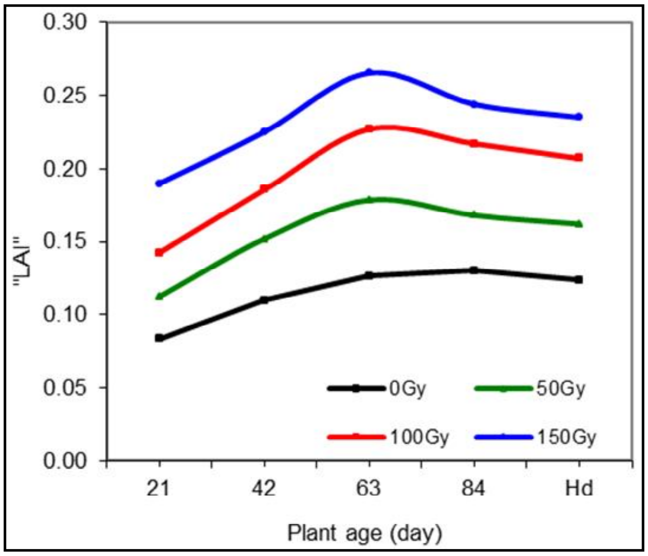

Fig. 6: “LAI” at different γ_d, Vs. plant age (day).

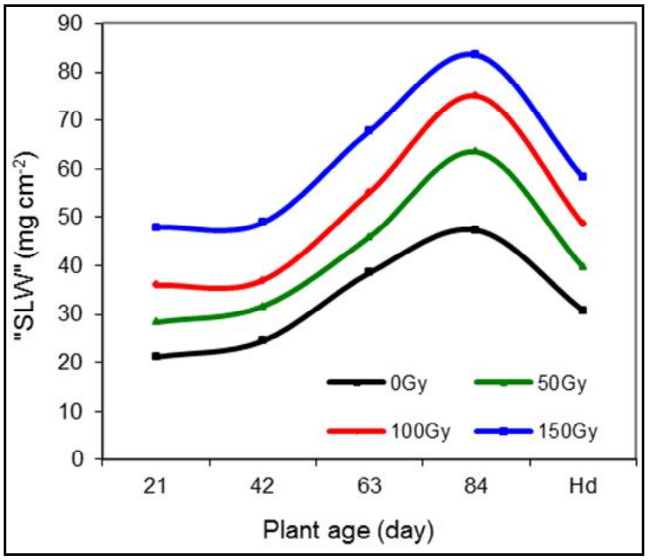

Fig. 7: “SLW” at different γ_d Vs. plant age (day).

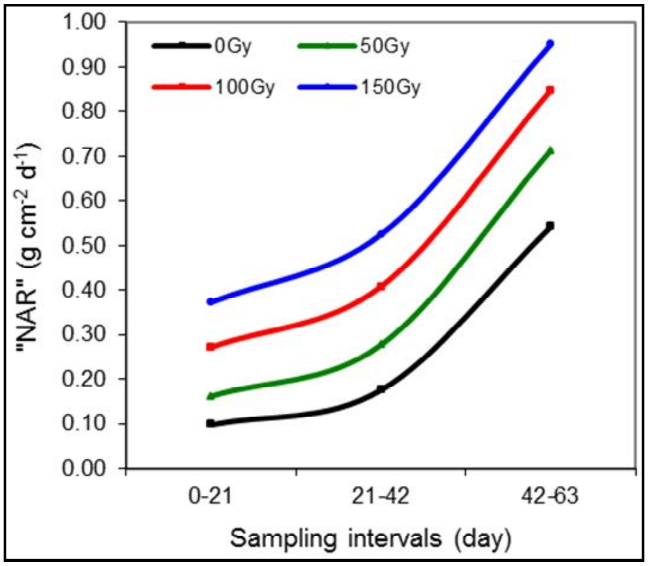

Fig. 8: “NAR” at different γ_d, Vs. sampling intervals (day).

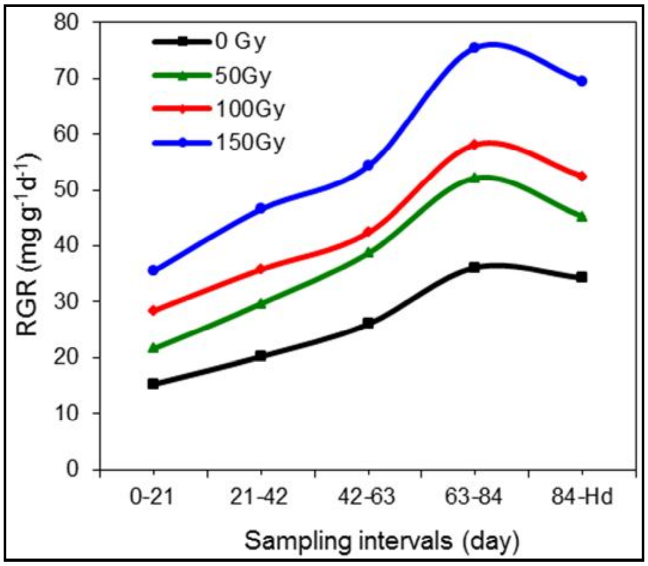

Fig. 9: “RGR” at different γ_d Vs. sampling intervals (day).

to 84-Hd).

Fig. 10 and 11. shows the increases in nodule parameters (NNP “^{1}.plant^{-1}” and NDM “mg plant^{-1}”) by progress plant age from 30 to 60 DAP, with different g_d. The highest value of NNP 124, 133, and 171^1.plant^{-1}., were recorded at PSI with 150Gy., on 30, 45 and 60 DAP, respectively. By augment plant age from 30 to 60 DAP, max. rate of NNP occur at control treatment (63.23%) followed by PSI with 100Gy., (42.15%), 150Gy., (37.9%) and 50Gy., (35.48%). NNP had direct relations with γ_d, at different plant age. Where, these relations were as followed: NNP $\approx 68.15\ \gamma_d^{\ 0.4124}$., $\approx 85.428\ \gamma_d^{\ 0.321}$., and $\approx 95.282\ e^{0.1437}\gamma_d$., at 30, 45 and 60 DAP, respectively. Similar behaviors were observed with NDM except at PSI with 150Gy. Where, NDM had improvement along with plant age by increasing γ_d, from 0 to 100Gy., and decrease thereafter at 150Gy. The highest value of NDM 1.14, 1.31., and 1.45 mg plant^{-1}., were recorded at PSI with 100Gy., at 30, 45, and 60 DAP, respectively. By increasing γ_d, from 0 to 100 Gy., max. rate of NDM scored at 45 DAP., (77.03%) followed by at 30 DAP., (70.15%), and at 60 DAP., (47.96%). Thereafter, increasing g_d from 100 to 150Gy., tended to decrease about 11.76, 9.16, and 5.07% at 30, 45., and 60 DAP., respectively. The relations between NDM and plant age at different g-dose were as followed: NDM $\approx 0.5375\ e^{0.1901(DAP)}$., ≈ 0.21 (DAP) + 0.75., ≈ 0.155 (DAP) + 0.99., and ≈ 0.18 (DAP) + 0.84., at 0, 50, 100 and 150Gy., respectively. These results were in agreement with this of Zaied *et al*., 2005., conversely with Farag *et al*., 2014., who mention that, nodulation parameters were recorded highest values with γ_d, 30Gy., while the irradiation dose 60Gy., ranked last in faba bean.

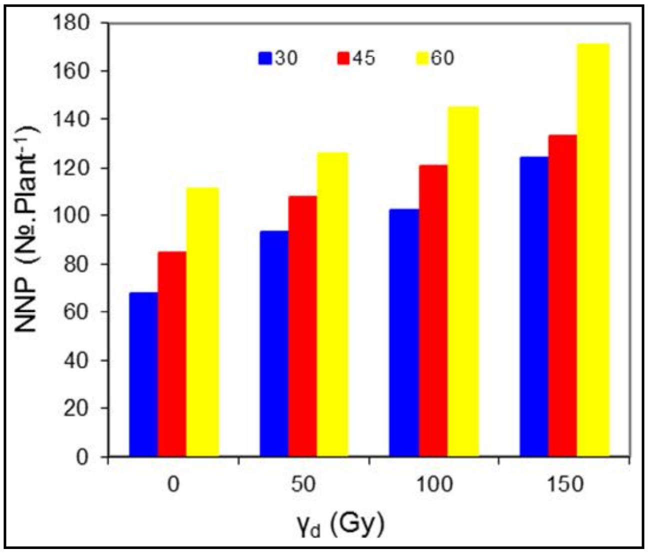

Fig. 10: “NNP” at different plant age Vs. γ_d.

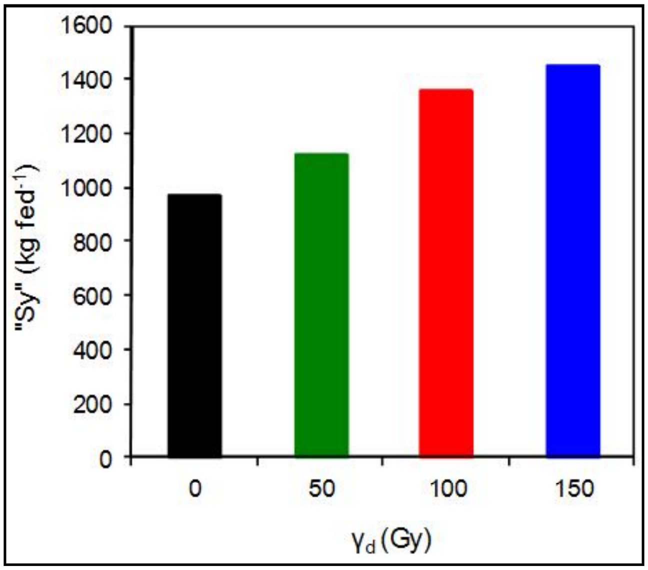

Fig. 12: “Sy” Vs. γ_d.

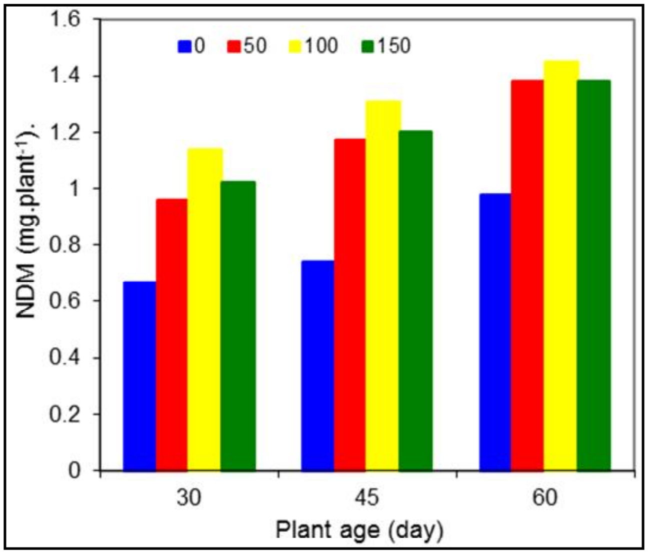

Fig. 11: “NDM” at different γ_d. Vs. plant age.

Results of seed yield “Sy” (kg fed^{-1}) as influenced by g_d, are presented in Fig. 12. The increase in “Sy” was more pronounced and significantly affect with pre-sowing seeds irradiation with 50, 100 and 150Gy., compared with which produced from control. Where, Sy had a positive linear relation with g_d. It was increase about 15.13, 33.78, and 35.15%., by increasing gamma dose from 0 to 50, 50 to 100 and 100 to 150 Gy., respectively. Farag and Abd El- Hameed, 2013., emphasized the importance of gamma irradiation in meliorating seed yield.

Discussion

Seeds store available material and energy for initial growth, in cotyledons. Low dose of γ- irradiation plus potential energy for initial growth inside seeds, stimulate RNA activation, protein synthesis, cell rate division by enzymatic activation, and awaking of the young embryo, during germination early stage. These boosting were observed by (Melki and Dahmani, 2009). Cell cycle arrest in the G2/M phase during somatic cell division and/or to a variety of damages in the entire genome, responsible for GP reduction with high-dose of gamma irradiation (Preuss and Britt, 2003). Where, Irradiation ionizes the atoms in meristematic tissues of the seeds by removing electrons from atoms, causing damages the bases of DNA, by broken chromosome strands, change their structure, and aberrations chromosomal frequency. Therefore, changes of the ascorbic acid content, auxin destruction, and another growth regulator. These could induce the inhibition of plant germination, and SSP (Muralidharan and Rajendran, 2013). A same augment behavior was observing in SG for irradiated seeds with gamma rays by Kumar and Mishar (2004) in okra seeds. This was maybe due to that short –wave photons (i.e., gamma rays) were more energetic, and had a stronger effect on the surface of plant cells, than visible light photons (> 400nm) causing ultimate breakdown of the seed coating allowing the germination to accrue (Aynehb and Afsharinafar, 2012).

With constant sample area (about 500cm^2., in this study), leaf numbers and leaf area (length and width), are two main factors effecting on leaf area index. Low dose till 100 Gy., had a stimulatory effect on both of them, by enhance growth regulator production, kinetin, which stimulating cell division rate and produced highest leaf area and leaves number. This increase persists in increased photosynthesis (Asare *et al*., 2017). Meanwhile, high dose of gamma irradiation adversely reduced the leaves number and had inhibitory effects on length and width of leaves, due to reduced endogenous growth regulators level, cytokinins, as a result of breakdown or synthesis lack (Kiong *et al*., 2008). Low LAI at start and end of growth season seemingly attribute to leaves senescent and scattering specifically those located at lower canopy layers.

The harmful effect of the gamma rays was mainly related to changes in the photosynthesis and the dark respiration. These were agree with Stoeva, 2002, who resulted that by increasing gamma dose from 0 to 80 or 100Gy, lead to decrease specific leaf area “SLA” (cm^2 g^{-1}) about 15 to 18%., in peas plant. Whereas, specific leaf weight “SLW” (g cm^{-2}) is reciprocal to “SLA”. Hence, gamma dose had a stimulate effect on SLW. On the other word, increasing gamma dose tended to leaf became more thicker, having more mesophyll cells, with higher densities of chlorophyll per unit area, and hence have greater photosynthetic and high values of dry matter content, (Aniya and Herzog, 2004).

The higher changes in the NAR parameter, characterizing the net assimilation rate, suggested that the leaves were more efficient in producing dry matter. NAR had negative correlation with SLA (Villar *et al*., 1998). Subsequently, this relation is positive with SLW. Where, thicker leaf had great rate of photosynthetic. This may explained increasing NAR values by increase gamma dose. Meanwhile, NAR values increase with plant age as a result of weeding resistance program. NAR and LAR had positive and negative correlations with RGR (Villar *et al*., 1998). Whereas, it had a positive correlation with SLW. Therefore, the stimulated effects for gamma doses on them reverberate positively on RGR. Where, by increasing gamma dose RGR tended to increase also.

At low doses of radiation (till 100Gy) tended to convers trytophan to IAA. Increasing IAA (indol acetic acid) production augment root dry weight, (area and length), lateral number and adventitious roots and root length. While, rhizobia grow in the rhizosphere of the host (root hair), establish contact with each other at the growing tip surface of root hair, and root hair curls to trap a small number of bacteria, induction of rhizobial nodulation gens by plant exudates and IAA excreted by bacteria on their cultures and increasing nodules parameters (number and weight). (Migahid, 1959, and Wang *et al*., 2012).

Furthermore, radiation till 100Gy., promote cell respiration, production of reproductive structures, led to increase both of leaves number, LAI and SLW, having more mesophyll cells, with higher densities of chlorophyll per unit area (Ch- a, and b), and hence have greater photosynthetic tended to higher growth, early maturation, and accelerated development (Badr *et al*., 2014), and having high values of dry matter. Also, it augments root area, resulting in increase in nutrient (NPK) uptake by plant, thereby increasing both plant growth and seed yields). The positive and stimulatory effects on seed yield were confirmed with Rhaman Din *et al*., 2010.

Conclusion

Gamma ray has stimulatory significant effects on germination indices. For cultivar under studied $LD_{50} \leq$ 200Gy. All germination indices *i.e*., GP, SSP, MGT, MDG, GI, GRI, and CVG, were increase by increasing g_d from 0 to 150Gy. Moreover, growth parameters LAI, SLW, NAR, RGR, followed similar patterns, and affected significantly by γ_d. Also, nodulation parameters “NNP” (^{1}.plant^{-1}) and “NDM”(mg plant^{-1}) had a direct proportion with gamma dose at different plant age. These relations were; $NNP \approx 68.15\ \gamma_d^{\ 0.4124}$., $\approx 85.428\ \gamma_d^{\ 0.321}$., and $\approx 95.282\ e^{0.1437}\gamma_d$., at 30, 45, and 60 DAP, respectively. As well as, $NDM \approx 0.5375\ e^{0.1901(DAP)}$., $\approx 0.21\ (DAP) +$

0.75., ≈ 0.155 (DAP) + 0.99., and ≈ 0.18 (DAP) + 0.84., at control and PSI with 50, 100, and 150Gy.,respectively. The increase in "Sy" was more pronounced and significantly affect with pre-sowing seeds irradiation with 50,100 and 150Gy., compared with which produced from control. Where, it had a positive linear relation with γ_d.

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