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Agro-Morphological Evaluation of Gamma Irradiated Oil Palm (*Elaeis guineensis*, Jacq.) M_2 Population at the Nursery Stage

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Abstract Morphological characterization is one of the steps that are considered important in the description and classification of cultivated crops. A field evaluation of gamma irradiated oil palm second generation (M_2) progenies was carried out at Council for Scientific and Industrial Research – Oil Palm Research Institute (CSIR-OPRI), Kusi, Ghana. This experiment aimed to determine the agro-morphological performances of M_2 population at the nursery stage. The entries were made up of eight (8) M_2 progenies and a control, using randomized complete block design with three replications. Data were collected on plant height, number of leaves, leaf area, butt circumference, chlorophyll content of the leaves and stomatal conductance. All the characters studied exhibited significant ($P < 0.05$) variability among genotypes. The mean value (58.45 cm) of plant height for all the M_2 progenies studied was 24% lower than that of the check (76.86 cm) and this is an indication of suspected dwarf trait which can be useful in crop improvement programmes. Progeny 14 performed significantly better in leaf production (9.60) and butt circumference (13.24 cm). The performance of progeny 15 was high with respect to leaf area (2135 cm^2) and chlorophyll content (32.93 ug/g) while progeny 16 exhibited low stomatal conductance (5.90 m^2s/mol), an indication of low transpiration rate and possible drought traits could be exploited in the development of drought tolerant materials.

Keywords Agro-Morphological, Gamma Irradiation, Oil Palm, Progenies

1. Introduction

Oil palm (*Elaeis guineensis*, Jacq.) is by far the most productive oil crop and alone is capable of fulfilling the large and growing world demand for vegetable oils that is estimated to reach 240 million tons by 2050 [4,19]. Oil palm has contributed substantially to economic improvements which have been reported by the producing countries. Dramatic expansion in cultivation has been experienced in recent times and forms a major habitat in the tropics [26]. A major proportion of the reported expansion has however occurred at the expense of tropical forest [21].

Irrespective of the socio-economic benefits of the oil palm, the genetic base of the crop is narrow stemming from

Deli duras derived from the four palms introduced in 1848 in the Bogor Botanical Gardens in Java, Indonesia which are often used as the female parents during hybridization programmes. The genetic base of oil palm which is restricted and coupled with breeding cycle which takes a long period (10–12 years) accounts for slow genetic advancement from one cycle of selection to the other, retarding the improvement of the oil palm crop [3,18]. Novel variation approach should be introduced into the breeding programmes to promote selection of Oil Palm with optimum yield and other traits of interest. Induced mutations, soma-clonal variations, transgenics, hybridization and germplasm can all introduce variations in a breeding population [33]. Aside from leveraging the genetic resources, introduced concept of transformative technologies provides important tools that enhance further research work. The improvement of these technologies over time and good application in the plant system reported, will enhance advancement of crop commodities [16,17,27].

Mutation induction has been reported to accelerate breeding in crop species and enhance novel traits development. Mutation in crops significantly promotes genetic variability and accelerates breeding programmes. Induced mutation by physical or chemical mutagens is one of the suitable approaches to create variation in plant breeding [7,11,28]. Induced mutants are noted to have characters that are superior such as lodging resistance, short stature, disease resistance, increased nitrogen fixation and improved oil quality [28]. Low doses of gamma irradiation led to about 14% increase in oil palm seed germination and a reduction of about 31.01% in plant height in recurrent irradiation (M_2M_1) population and suggested the potential of introducing the putative dwarf trait observed in future breeding programmes [6]. Thus induced mutation through gamma irradiation may be a useful tool in creating variability in oil palm. Mutation creates new variants (alleles) of genes and is a quick way of creating variability for crop improvement through breeding and selection. Oil Palm is one of the few major crop species, and the only oil crop yet to be improved by mutation breeding [23]. Plant growth study is a quantitative method employed to describe and interpret the performance of the whole plant system grown under natural, semi-natural or controlled conditions accompanied by a quantitative change in biomass [12,13]. Plant growth analysis is still basic and various techniques are needed to evaluate different physiological stages during vegetative and regenerative periods [24]. Information regarding oil palm research work on plant agro-morphological traits of irradiated materials at the nursery stage is limited since the focus has mostly been on yield and its related traits in non-irradiated palms. Morphological characterization is one of the steps that are considered important in the area of cultivated crop description and classification [22]. This study aimed to determine the agro-morphological performance of irradiated oil palm M_2 (second generation) population at the nursery stage and to determine whether the observed variation will reflect future field performance which will impact positively on fresh fruit bunch (ffb) production.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted from June 2015 to May 2016 at OPRI, Kusi (0.6.00 N, 001.45W) within the Denkyembour district which falls within the semi deciduous forest zone of Ghana.

2.2. Plant Materials

Seeds for this experiment were obtained from the Crop Improvement Division of OPRI in Ghana. The progenies of gamma irradiated pollen and seed at a dosage ranging from 10-50 Gray (Gy) were used for the experiment. Eight (8) M_2 oil palm seedling populations obtained from previously selfed M_1 population obtained from irradiated seeds and pollen and a check (Table 1) also were used. Details of progeny, irradiation dosages and irradiated material are presented in Table 1. The experiment was laid out in randomized complete block design with three replications.

Table 1. M_1 irradiated materials selfed to produce the M_2 used for the experiment

Progeny (P)	Material Irradiated	Radiation Dose Gray (Gy)	Crosses	
			Female	Male
13	Seed	10	852.802T	852.121D
14	Seed	20	852.802T	852.121D
15	Seed	30	852.802T	852.121D
16	Seed	50	852.802T	852.121D
34	Pollen	50	K1:4843D	851.805P
35	Pollen	10	K1.3981D	851.805P
36	Pollen	15	K1.2520D	851.805P
37	Pollen	10	K1.1988D	851.805P
38 (check)	Seed	0	5.37D	4.17P

T- Tenera, D- Dura and P- Pisifera

2.3. Data Collection

Data collection started when seedlings were five (5) months old and data were taken on a monthly basis for eight months (5 to 12 months after planting). Ten plants per plot were randomly selected and tagged for data collection for each of the growth and physiological parameters studied. These parameters observed are as follows:

2.3.1. Plant Height

Data on plant height was taken with a tape measure from the soil level in the polybag to the tip of the longest leaf at monthly interval from 5 to 12 months after planting.

2.3.2. Number of Leaves

The number of leaves per plant was determined by counting the number of leaves on the randomly selected seedlings on monthly basis.

2.3.3. Leaf Area

Leaf area measurement was carried out using a non-destructive method. Sampled plants per plot were randomly selected and leaf length and width measured using a ruler and leaf area estimated using the formula used by [6].

2.3.4. Butt Circumference

The measurement of butt circumference was carried out with Vernier caliper at two places on the butt 0.5 cm above the soil level. The measurement was carried out at monthly interval from 5 to 8 months and butt circumference calculated by the formula $\boldsymbol{\pi} d$. Where $\boldsymbol{\pi}$ was taken as 3.14 and d is the average diameter measured

2.3.5. Chlorophyll Content

The chlorophyll content was measured using a chlorophyll meter (SPAD 502 Plus) by following the procedure used by previous authors. Intact leaf samples from frond number 3 were used for this determination. The leaf blade was surface cleaned with distilled water and wiped. The leaf was placed between the arm and the sensor of the chlorophyll meter and 3 random spots around the mid-point of each leaf blade were measured for chlorophyll content [6,30].

2.3.6. Stomatal Conductance

Stomatal conductance was measured on the upper (adaxial) surfaces of the leaves using Leaf Porometer (SC-1) close to solar noon, from 11:00 am to 2:00 pm as described by previous authors [6]. The randomly selected leaves were young, intact, green and fully exposed to the sunlight. The instrument is automated and calculates the stomatal conductance when leaf is clipped to the sensor.

2.4. Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using GENSTAT (Teaching edition, version 12) software and the treatment means separated using the least significant difference (LSD) test at 5% significant level.

3. Results and Discussion

There were significant differences ($P < 0.05$) among the M_2 progenies for plant height, number of leaves, leaf area and butt circumference. The traits, chlorophyll content and stomatal conductance were not significant (Table 2). The mean, maximum, minimum and coefficient of variation (CV) values for all the characters studied are presented in Table 3.

Table 2. Mean square of quantitative traits among M_2 populations

Source of variation	Degree of freedom	Mean square					
		PH	NL	LA	BC	CC	SC
Replication	2	47.90	0.07	12445	0.11	26.95	5.80
Progeny	8	148.90**	0.99**	156210*	2.79*	15.83^{ns}	7.19^{ns}
Residual	16	5.29	0.15	60437	0.56	17.91	5.19
Total	26	52.75	0.40	86214	1.21	17.96	5.86

Note: ** Significant at P 0.01%, * Significant at 0.05% and ns: not significant. PH- plant height, NL- number of leaves, LA- leaf area, BC- Butt circumference, CC- chlorophyll content, and SC- stomatal conductance

Table 3. Traits and their means, maximum, minimum and CV of M_2 population

Trait	Mean	Maximum	Minimum	CV
Plant Height (cm)	60.45	76.86	51.64	3.80
Number of Leaves	8.34	9.60	7.58	4.71
Leaf Area (cm^2)	1791	2135	1549	13.73
Butt Circumference (cm)	11.70	13.24	10.10	6.37
Chlorophyll Content (ug/g)	30.42	32.93	25.60	13.91
Stomatal Conductance (m^2s/mol)	7.43	10.57	5.90	30.68

Note: CV= coefficient of variation

3.1. Plant Height

Plant height ranged from 51.64 to 76.86 cm with a mean of 60.45 cm. Progeny 38 (check) recorded the highest plant height of 76.86 cm which was significantly ($P < 0.05$) different from the rest of the progenies. The lowest plant height was recorded in progeny 34 and was not significantly different from progenies 35, 36 and 37 (Figure 1). Plant height obtained from pollen irradiated progenies decreased significantly ($P \leq 0.05$) with increasing doses of gamma irradiation and this was in conformity with other authors [1]. The mean value (58.45 cm) of plant height for all the M_2 progenies studied was about 24% lower than the check (76.86 cm). The decreased in plant height may be due to disruption during cell division and cell elongation as a result of radiation treatment [1]. Reduction in growth has been attributed to destruction of auxin, changes in the content of ascorbic acid, and biochemical and physiological disturbances [1,10]. Earlier work reported about 31.01% reduction in plant height in recurrent irradiation population and this probably has the potential of introducing dwarf traits into breeding programmes [6].

3.2. Number of Leaves

Number of leaves ranged from 7.58 – 9.60 with a mean of 8.34. The highest number of leaves was recorded in progeny 14 (9.60) (Figure 2). This study revealed a relative reduction in number of leaves of gamma treated progenies compared to the check and this result is not in agreement with earlier work where higher leaf production was observed in irradiated materials [1]. However, other authors reported a reduction in leaf production of gamma irradiated treatment above 10 Gy [9]. Gamma radiation causes chromosomal aberration of plants and can affect plant growth potential of the leaves [19].

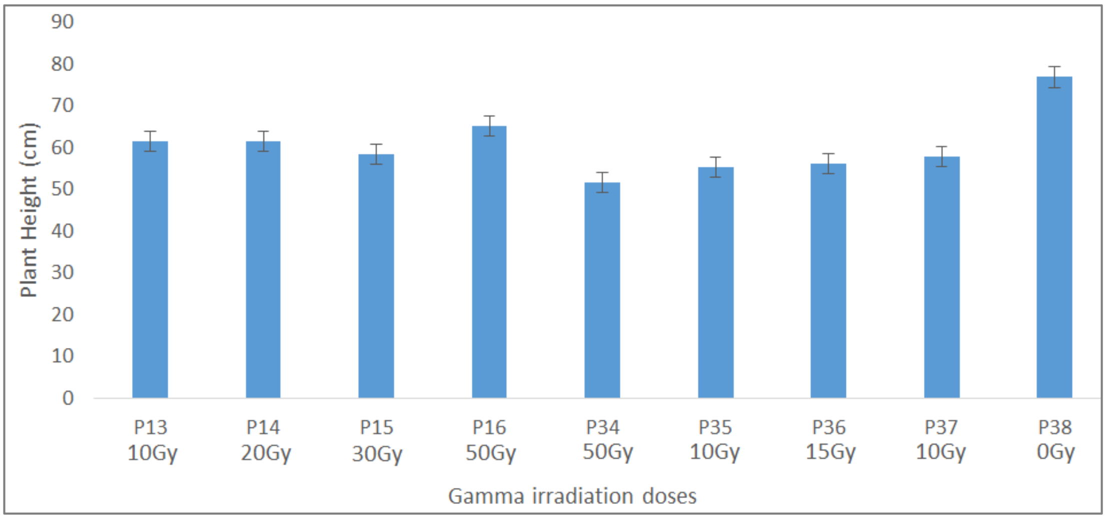

Figure 1. Effects of gamma irradiation on plant height of M_2 populations at the nursery stage

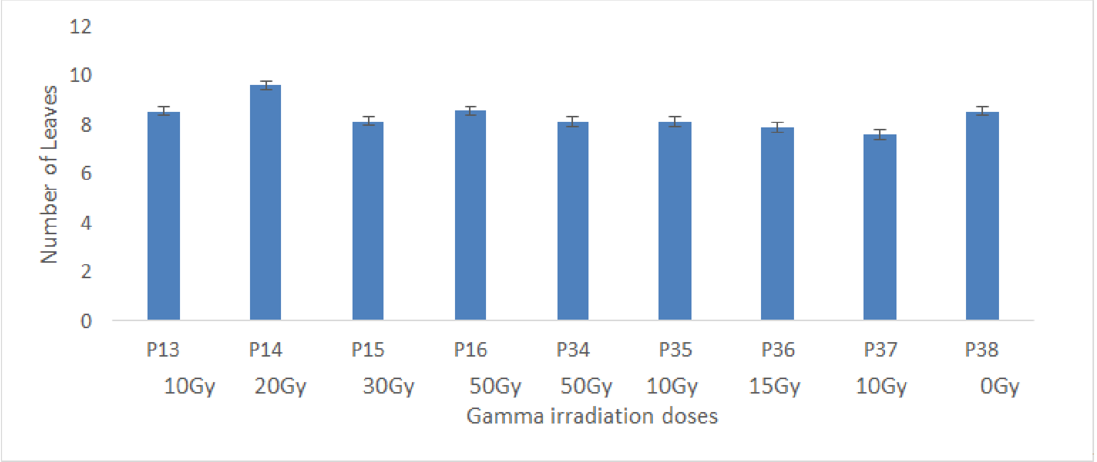

Figure 2. Effects of gamma irradiation on number of leaves of M_2 populations at the nursery stage

3.3. Leaf Area

The range for leaf area was from 1549 to 2135 cm^2 with a mean of 1791 cm^2. Among the irradiated treatments, for seed irradiated progenies, increasing doses resulted in an increasing leaf area up to 30 Gy after which leaf area declined at 50 Gy. Irradiated pollen also had the least leaf area at 50 Gy. Progeny15 exhibited the highest leaf area (2135 cm^2) and was significantly ($P < 0.05$) different from the rest of the progenies and that of the check (Figure 3). Progenies 13, 14, and 16 from seed irradiated source did not show any significant difference among them. Progenies 34, 35, 36 and 37 from pollen irradiated source also did not show significant difference. This study revealed a significant difference between the seed irradiated progenies and pollen irradiated progenies in leaf area and this might be due to the type of material subjected to gamma treatment. The induction of higher leaf area development by progeny 15 might therefore lead to higher yields in later years. Observation should continue for future performance. Nursery seedlings study indicated that leaf area could be used as selection criteria in 9-month-old seedlings as it correlates positively with yield [32]. The interception of light is determined by leaf area and leaf area is an important trait that determines crop productivity [14].

3.4. Butt Circumference

Significant ($P < 0.05$) difference was observed in butt circumference and ranged from 10.10 to 13.24 cm with a means of 11.70 cm. Progeny 14 recorded the highest butt circumference of 13.24 cm (Figure 4). Progeny 16 and the check also had similar results. Previous work observed no significant difference in butt circumference from 4 – 7 months after planting in mutagenic experiment to determine the effects of gamma irradiation on germination and growth of oil palm. Non-significant difference was attributed to the retarded growth at the initial stage of the plant. However, significant difference was observed from 8 – 12 months which is in conformity with the findings of the current study [6]. Similar results in improving oil palm seedling growth using bio-stimulants were also reported [5]. This might probably be due to the seedling ability to overcome any inhibitory substance that retards growth and seedling development. Nursery seedlings study indicated that butt diameter could be used as selection criteria in 9-month-old seedlings as it correlates positively with yield [32].

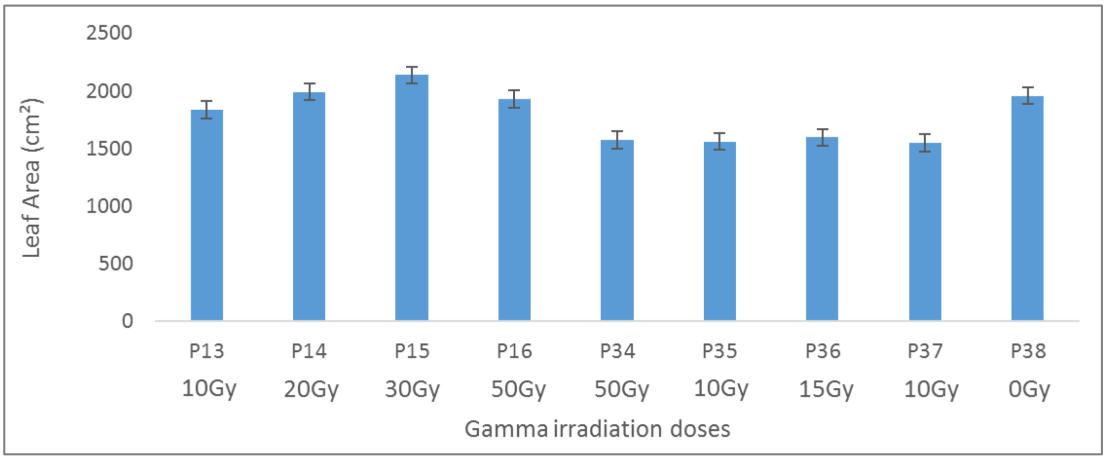

Figure 3. Effects of gamma irradiation on leaf area of M_2 populations at the nursery stage

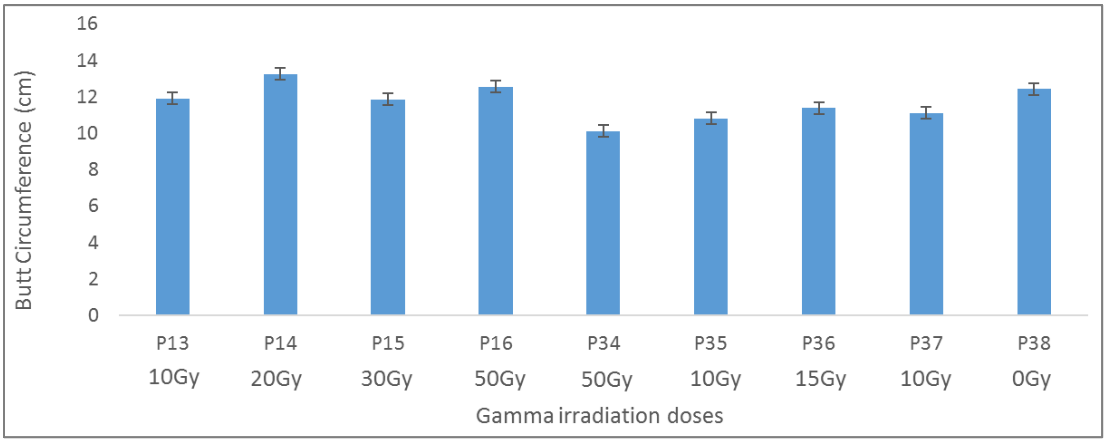

Figure 4. Effects of gamma irradiation on butt circumference of M_2 populations at the nursery stage

3.5. Chlorophyll Content

The green photosynthetic pigment that absorbs sunlight is the chlorophyll and energy is transferred to the reaction center of the photosystems. Photosynthesis is a major condition in establishing plant assimilates and it is determined by available CO_2 and H_2O while the synthesis of carbohydrates is influenced by light and chlorophyll [2]. Chlorophyll content ranged from 25.60 to 32.93 ug/g with a mean of 30. 42 ug/g. The highest chlorophyll content was recorded in progeny 15 (32.93 ug/g) (Figure 5). Similar result was observed in progeny 16. The higher doses (30 Gray and 50 Gray) from the seed irradiated progenies recorded an increase in chlorophyll content. This result is in accordance with some previous studies that reported a decrease in chlorophyll content at lower irradiation doses and an increase at higher doses in *Lablab purpureus* L, *Vigna unguiculata* L. and *Phaseolus vulgaris* L. [31]. However, reduction in mean chlorophyll content at higher gamma doses has also been reported. A number of studies have considered chlorophyll content as a reliable drought tolerance indicator [2].

3.6. Stomatal Conductance

Stomatal conductance also ranged from 5.90 to 10.57 m^2s/mol with a mean of 7.43 m^2s/mol. Progeny 14 (10.57 m^2s/mol) had the highest stomatal conductance which was significantly different from the rest of the progenies and that of the check. Progeny 16 (5.90 m^2s/mol) exhibited the lowest stomatal conductance and was not significantly different from progenies 13, 15 and 34 (Figure 6). These progenies are characterized by low transpiration rate and may be candidates for the development of drought tolerant materials. Screening of M_2 population for drought tolerance may throw more light on these preliminary observations. Stomatal conductance determines the rate of transpiration and plays an important role in regulating plant water balance. Cell expansion and plant growth rate are also reduced by stomata closure and thus lead to a significant decline of biomass and yield. Stomatal regulation is a key process involved in the maintenance of photosynthetic capacity in plants under stress conditions [20,25]. Observation of the vegetative growth and field performance (yield) of these M_2 progenies in later years may confirm these findings made by previous authors.

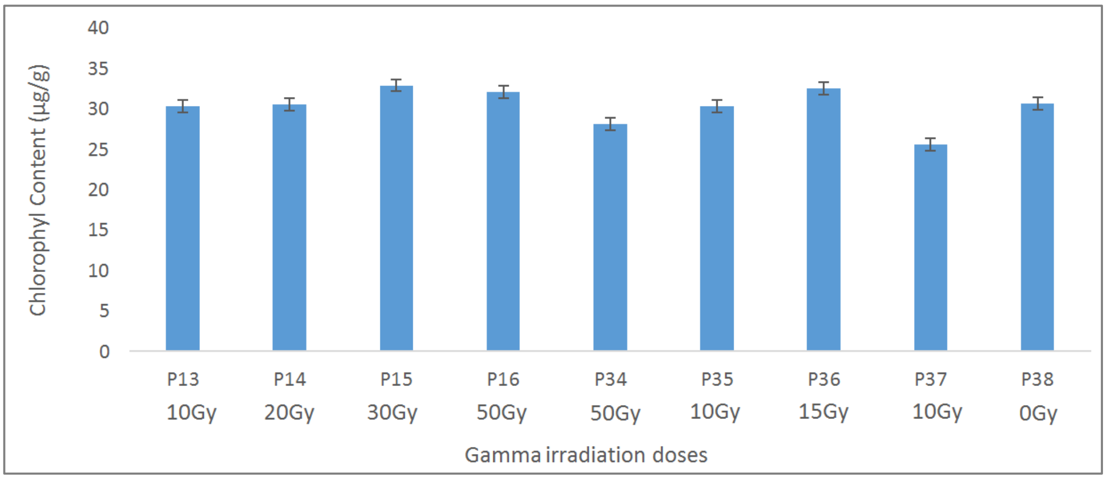

Figure 5. Effects of gamma irradiation on chlorophyll content of M_2 populations at the nursery stage

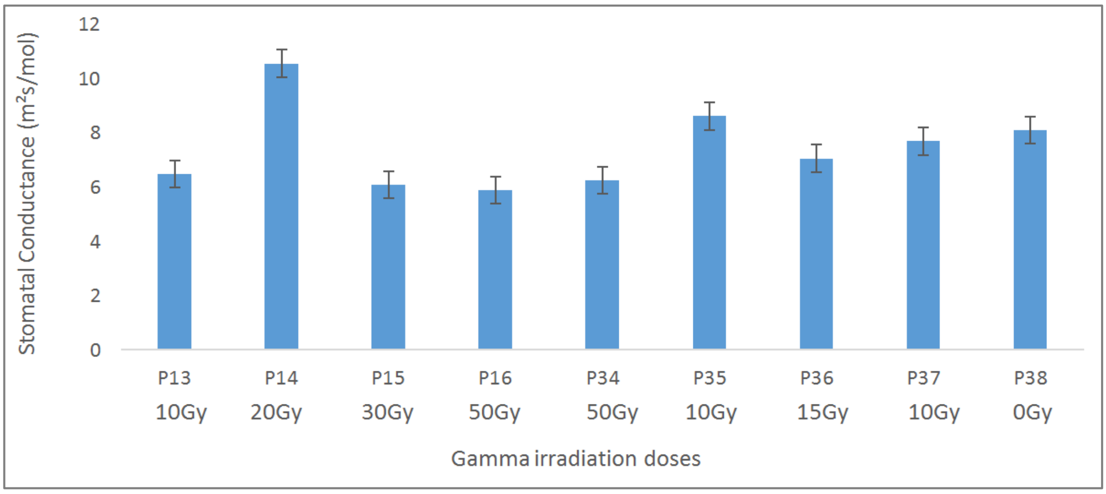

Figure 6. Effects of gamma irradiation on stomatal conductance of M_2 populations at the nursery stage

4. Conclusions

Exposure of oil palm seeds and pollen to gamma irradiation elicited various responses in the agro-morphological parameters studied in the M_2 populations. Progeny 14 performed better in the number of leaves (9.60) and butt circumference (13.24 cm). The performance of progeny 15 was also high in leaf area (2135 cm^2) and chlorophyll content (32.93 ug/g). The mean value (58.45 cm) of plant height for all the M_2 progenies studied was about 24% lower than the check (76.86 cm) and this is probably an indication of dwarf trait which can be useful in crop improvement programmes. Progeny 16 also exhibited low stomatal conductance (5.90 m^2s/mol) which is probably an indication of low transpiration rate and may be a candidate for the development of drought tolerant planting materials. This work amply demonstrates the potential use of gamma irradiation in creating useful variability in important agro-morphological traits in the oil palm that could lead to the development of improved planting materials (high yielding, environmentally adapted) as it pertains in other field crops. It will also serve as a guide or reference material for future work on oil palm mutation breeding.

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