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Photosynthetic Pigments and Stomata Characteristics of Cowpea (*Vigna sinensis* savi) under the Effect of X-Ray Radiation

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ABSTRACT

This study was conducted in the field and laboratories of the faculty of science and health-Koya university by exposing the seeds of cowpea plant (*Vigna sinensis* Savi) var. California black-eye to X-ray radiation in two different locations (In target or 30 cm out of target) inside the radiation chamber, for four different exposure times (0, 5, 10, or 20 min), to study the effect on some characteristics of seedling components. Results show that the exposure location to X-ray had non-significant effects on cowpea leaves content of photosynthetic pigments, whereas each of time of exposure with interaction between location and time of exposure had significant effects on chlorophyll a, total chlorophylls, and total carotenoids pigments. Regarding the X-ray effects on stomata characteristics, the results detect that there were non-significant differences between the location of exposure on stomata number on abaxial leaves surfaces and stomata length on adaxial leaves surfaces, whereas a significant effects on number of stomata on the adaxial leaves surfaces, abaxial stomata length, abaxial, and adaxial stomata width were detect. Exposing cowpea seeds to X-ray radiation in the target of the radiation source increased significantly stem and leave dry matter percent compared with the one out of the target location, whereas increasing the time of exposure decreased the percent of dry matter of stem and leaves. It is concluded that exposing cowpea seeds to X-ray leads to changes in photosynthetic pigments, stomata characteristics, and plant dry matter content.

Index Terms: *Vigna sinensis* savi, X-ray Radiation, Pigments, Stomata Traits

1. INTRODUCTION

In the field of agriculture, many practices particularly the using of chemicals are applied for improving crops quality and quantity, however, although their positive effects, these applications are not empty of undesirable effects on environment, public health, and plant growth. Using modern biotechnological approaches, including, electricity current, laser, magnetic field, high voltage, ultraviolet and radiation with gamma or X-ray on different plants material are gaining interest to develop plants growth and yield, and characterized by cheapness and safety on health and environment, therefore the scientists try to make this century a biophysical century, where most of the physical factors depend on increasing energy balance and increase material transport through membranes for improving the growth and the development of crops [1]-[3].

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Ionizing radiations are those have wavelengths <100 nm [4]. These radiations are charged high-energy particles (high-energy photons and electrons). Two types of ionizing radiations there are: Gamma radiations and X-rays, the first is emitted from inside the nucleus, whereas X-ray is radiated from outside the nucleus [5]. There are many applications of X-ray radiation in different fields of plant studies, for example Panchal *et al.* [6] used X-ray for imaging of inner features of a seed sample to identify unseen defects or contaminants. Other studies were conducted to investigate the effects of X irradiation on physiological characteristics of different plants, such; Rezk *et al.* [7] found that low dose of X-ray 5 Gray (Gy) caused increasing in all morphological criteria, total photosynthesis pigments, enzymatic and non-enzymatic antioxidants significantly in two genotypes of okra plants as compared with control treatments, while the doses (higher than 5 Gy) caused a considerable decreased in the studied parameters. Similarly, Singh [8] study shows promoting in chlorophyll development for 60 s X-ray pretreated as it compared to 90 and 120 s pre-treatment for seeds of *Cicer arietinum*, *Vigna radiata*, *Vigna mungo* and *Vicia faba* plants. Dhamgaye *et al.* [9] irradiated seeds of *Phaseolus vulgaris* cv. Rajmah using Synchroton X-ray Beam at 0.5–10 Gy, the overall growth of 10 days old seedlings raised from irradiated seeds was substantially reduced at irradiation doses of 2 and 5 Gy. Same authors Dhamgaye *et al.* [10] irradiated seeds of *P. vulgaris* cv. Rajmah using Synchrotron X Ray at doses of 1, 10, and 20 Gray where, the percent of relative water and protein content was significantly decreased at 10 and 20 Gy dose in 4–8 days old seedling, and a decrease in photosynthesis pigments chlorophyll and carotenoids content is observed in shoot tissue when 1 and 10 Gy where used. Mortazavi *et al.* [11] accelerated the growth of newly grown plants of *P. vulgaris* (Pinto) by irradiated them with X-rays for 6 days. Arena *et al.* [12] found that exposure of dwarf bean (*P. vulgaris* L.) plants to different doses of X-rays (0.3, 10, 50, and 100 Gy) showed that young leaves exhibited a reduction of area and an increase in specific mass and dry matter content. At higher doses of X-rays (50 and 100 Gy) total chlorophyll (a+b) and carotenoid (xanthophylls + carotenoids) content were significantly lower ($P < 0.01$) compared to lower doses and in control leaves. Significant reduction in transpiration was detected in *V. faba* irradiated by X-ray, this reduction was associated with inhibition of stomatal opening from the 9th to 16th day after irradiation. The osmotic pressure of epidermal cells in irradiated plants appeared to be slightly higher than that of epidermal cells of non-irradiated plants. However, the slight osmotic pressure changes of epidermal cells in irradiated plants did not appear to be a major factor contributing to inhibition of stomatal opening in irradiated plants under the growth conditions of the experiments [13].

The aim of this work was to investigate the effects of seed exposure to X-rays on some of the physiological properties of emerged cowpea plants, because these changes has subsequent effects on the photosynthetic activity and cause a direct effect on the agronomic features of the plant.

2. MATERIALS AND METHODS

2.1. Plant Materials and Studied Characteristics

This work was conducted in the Department of Biology/Koya University, Erbil-Iraq. The seeds of cowpea plant (*Vigna sinensis Savi*) var. California black-eye were exposed to a single dose of X-ray radiation by the XRD tube (from the Company of PANalytical B.V. Lelyweg1, the Netherlands) where the highest radiation level was less than 1 Sieverts/h measured at the tube surface. 20 seeds for each experimental unit were putted in the device source to exposed to X-ray at the advanced physics laboratory in physic department at same faculty. The experiment was conducted in complete randomize design (CRD) where the location of exposure considers as the first factor by exposure the seeds to X-rays either in the target point of the device or 30 cm from the target point in the base of the device chamber, whereas the times of exposure 0, 5, 10, or 20 min were considered as the second factor, where the time zero is considered as the control treatment used for each location.

After seeds were exposed to X-ray they planted in 5 kg. soil pots, because of an initial increase in photosynthesis rate during leaf expansion and followed by a decrease on maturation [14], at the end of the vegetative growth stage, fourth leaf of five plants from each experimental unit were taken, and the photosynthetic pigments chlorophylls a, chlorophyll b, total chlorophylls and total carotenoids were estimated as it mentioned in Lichtenthaler and Wellburn [15] were leaf material was collected and mixture ratio was 50 ml 80% acetone: 1 g leaves sample. Samples were grinded by mortar and pestle and filtered by filter paper, then extracts were placed in a 25 ml dark glass vial to avoid evaporation and photo-oxidation of pigments, after that the absorbance of the extract were measured by spectrophotometer at wave lengths 663, 646, and 470 nm. Each of chlorophyll a, b, and total carotenoids were estimated as follows:

$$\text{Chlorophyll a} = (12.21 * A663) - (2.81 * A646)$$

$$\text{Chlorophyll b} = (20.13 * A646) - (5.03 * A663)$$

Total carotenoids = (1000*A470 - 3.27*Chl a – 104* Chl b)/229

Where, A is Absorbance, chl. a = chlorophyll a (mg/L) and chl. b = chlorophyll b (mg/L).

For converting the concentration from mg/L to mg/g fresh weight, each value multiplied by (extraction volume/sample weight *1000), and total chlorophyll calculated from the summation of each chlorophyll a and chlorophyll b.

Total chlorophyll was determined by collecting each of chlorophyll a and chlorophyll b (10).

For stomata study, the lasting impressions method [16] was used. In this method, about one square centimeter of leaves surfaces was painted by a clear nail polish. After the nail polish was dried they were taped by a clear cellophane tape, and peels it out. The leaf impressions taped on slides and labeled as adaxial and abaxial surfaces then examined under ×40 by light microscope (DM 300, Leica Microsystems, China). Numbers of appeared stomata on lens field were counted for all adaxial and abaxial leaves surfaces. Stomata guard cells length and width of adaxial and abaxial leaves surfaces were calculated in micrometer (μm) with scaled ocular lens.

Because of the important of the percent of dry matter content as a result of the photosynthetic activity it determined for each of stem and leaves by dividing the stem or leaves dry weight by the stem or leaves fresh weight multiplying by 100 as it reported by Al-Sahaf [17].

2.2. The Statistical Analysis

The statistical analysis of the study conducted as a factorial experiment performed as CRD in three replications, analysis of variance was used for calculating the differences among each factor treatments and their interactions by using the SAS software. The test of Duncan's multiple comparison was used to estimate the main effects of treatments which were differ when the F-value was significant at $P \leq 0.05$ [18].

3. RESULTS AND DISCUSSION

From Table 1 results it is shown that location of exposure to X-ray had non-significant ($P > 0.05$) effects on leaves content of photosynthesis pigments of cowpea plant, whereas the time of exposure led to a significant ($P \leq 0.05$) effects on chlorophyll a and total chlorophylls, were the seeds that exposed to X-ray for 10 min increased chlorophyll a and total chlorophylls significantly ($P \leq 0.05$) to 2.64 and 5.42 mg/g fresh weight comparing to other exposure times. Results of interactions between locations and time of exposure revealed that exposure for 10 min out of target increased the content of chlorophyll a significantly to 3.01 mg/g fresh weight compared to other interaction treatments, and to 3.14 and 6.15 mg/g fresh weight for each of chlorophyll b and total chlorophylls compared with 5 min exposure out of target only, whereas same interaction increased total carotenoids content significantly to 1.15 mg/g fresh weight compared to 0.94 mg/g fresh weight for 20 min exposure out the target interaction treatment only. In general, ionizing radiation may have different effects on plant metabolism, growth and

TABLE 1: Effects of location and time of exposure cowpea seed to X-radiation, and their interactions on chlorophyll a, b, total chlorophylls and total carotenoids

Treatments	Chlorophyll (mg/g fresh weight) a	Chlorophyll (mg/g fresh weight) b	Total chlorophylls (mg/g fresh weight)	Total carotenoids (mg/g fresh weight)
Location of exposure				
In target (L1)	2.23[a]	2.33[a]	4.56[a]	1.05[a]
Out of target (L2)	2.32[a]	2.38[a]	4.70[a]	1.04[a]
Time of exposure (min)				
T0	2.20[b]	2.27[a]	4.47[ab]	1.07[a]
T5	2.11[b]	1.96[a]	4.07[b]	1.01[a]
T10	2.64[a]	2.78[a]	5.42[a]	1.09[a]
T20	2.16[b]	2.40[a]	4.56[ab]	0.99[a]
Interactions between location and exposure time				
L1×T0	2.20[b]	2.27[ab]	4.47[ab]	1.07[ab]
L1×T5	2.27[b]	2.27[ab]	4.54[ab]	1.02[ab]
L1×T10	2.27[b]	2.42[ab]	4.70[ab]	1.03[ab]
L1×T20	2.18[b]	2.34[ab]	4.52[ab]	1.05[ab]
L2×T0	2.20[b]	2.27[ab]	4.47[ab]	1.07[ab]
L2×T5	1.96[b]	1.65[b]	3.60[b]	0.99[ab]
L2×T10	3.01[a]	3.14[a]	6.15[a]	1.15[a]
L2×T20	2.13[b]	2.46[ab]	4.59[ab]	0.94[b]

Means that followed by same letters within column are differ non-significantly at $P \leq 5\%$ according to the Duncan multiple range test reproduction, depending on radiation dose, plant species, developmental stage, and physiological traits [12]. Our results disagree with the Al-Enezi and Al-Khayri [19] results that suggested that photosynthesis pigments chlorophyll a and carotenoids are more sensitive to X-ray than chlorophyll b, whereas we found that chlorophyll b and total carotenoids were less sensitive to X-irradiation compared to chlorophyll a and total chlorophylls. Changes in photosynthetic pigments were studied by Arena *et al.* [12] whom confirmed that the decrease in the levels of X-ray (0.3 Gy) caused an increase in photosynthetic pigments in bean plants, whereas the high levels (50 and 100 Gy) caused a decrease in these pigments, these findings also agree with that of Rezk *et al.* [7] which recorded in two okra genotypes leaves, where the content of photosynthetic pigment improved significantly with increasing the doses of X-ray to 5 Gy comparing with untreated plants, also more increase in the radiation doses, encourage the reduction in photosynthetic pigments compared to the control plants. Changes in chlorophyll content as a response to X-ray is either toward an increase or a decrease direction, the increase may due to the increase in chlorophyll biosynthesis and/or delaying its degradation [20], whereas the decrease may due to pigment breakdown due to increase of reactive oxygen species [21] and changes in the chloroplast such chloroplast swelling, thylakoid dilation, and breakdown of chloroplast outer membrane [22].

Regarding X-radiation effects on the stomata characteristics it was shown that there were non-significant ($P > 0.05$) differences between the location of exposure on number of stomata on abaxial leaves surfaces and stomata length on adaxial surfaces of leaves (Table 2 and Figs. 1 and 2). The seeds exposed directly to the source of X-ray (in target) decreased number of stomata on the adaxial leaves surfaces to 148.33 stomata/mm^2, whereas abaxial stomata length increased to 11.08 micrometer and abaxial with adaxial stomata width also increased significantly to 7.00 and 7.58 micrometer, respectively, compared to 180.00 stomata/mm^2, 9.92, 5.42, and 5.50 micrometer for plants out of target. 10 min of seed exposure to X-ray increased stomata number on both abaxial and adaxial leaves surfaces compared to other exposure times except the control treatment. Exposure time had non-significant ($P > 0.05$) effect on stomata length and width on abaxial leaves surfaces, whereas increasing time of exposure to 20 min increased the stomata length significantly ($P \leq 0.05$) compared to 5 and 10 min only, whereas it increased the stomata width significantly compared to all other treatments. From the results of interaction within location and time of exposure, it was clear from the results (Table 2), that treating seeds for 10 min in the X-ray target had the more significant effects for abaxial leaves surfaces in increasing stomata number to 540.00 stomata/mm^2, and the stomata length and width to 11.67 and 8.00

TABLE 2: Effects of seeds exposure to X-radiation on some characteristics of cowpea (*Vigna sinensis* Savi) plants stomata

Treatments	Stomata number/mm^2		Stomata length (micrometer)		Stomata width (micrometer)	
	Abaxial leaves surface	Adaxial leaves surface	Abaxial leaves surface	Adaxial leaves surface	Abaxial leaves surface	Adaxial leaves surface
Location of exposure						
In target (L1)	455.00^a	148.33^b	11.08^a	10.58^a	7.00^a	7.58^a
Out of target (L2)	455.83^a	180.00^a	9.92^b	11.67^a	5.42^b	5.50^b
Time of exposure (min)						
T0	526.67^a	176.67^{ab}	10.67^a	12.33^a	6.00^a	6.00^{bc}
T5	398.33^b	146.67^{bc}	10.33^a	9.67^b	6.17^a	5.17^c
T10	536.67^a	201.67^a	10.17^a	10.17^b	6.00^a	6.67^b
T20	360.00^b	131.67^c	10.83^a	12.33^a	6.67^a	8.33^a
Interactions between location and exposure time						
L1×T0	526.67^{ab}	176.67^{bc}	10.67^{ab}	12.33^{ab}	6.00^{abc}	6.00^{bc}
L1×T5	403.33^{bc}	143.33^{bc}	10.67^{ab}	10.33^{bcd}	7.33^{ab}	6.00^{bc}
L1×T10	540.00^a	156.67^{bc}	11.67^a	8.33d	8.00^a	8.67^a
L1×T20	350.00^c	116.67^c	11.33^a	11.33^{abc}	6.67^{ab}	9.67^a
L2×T0	526.67^{ab}	176.67^{bc}	10.67^{ab}	12.33^{ab}	6.00^{abc}	6.00^{bc}
L2×T5	393.33^c	150.00^{bc}	10.00^{ab}	9.00cd	5.00^{bc}	4.33^c
L2×T10	533.33^a	246.67^a	8.67b	12.00^{ab}	4.00^c	4.67^{cd}
L2×T20	370.00^c	146.67^{bc}	10.33^{ab}	13.33^a	6.67^{ab}	7.00^b

Means that followed by same letters within column are differ non-significantly at $P \leq 5\%$ according to the Duncan multiple range test

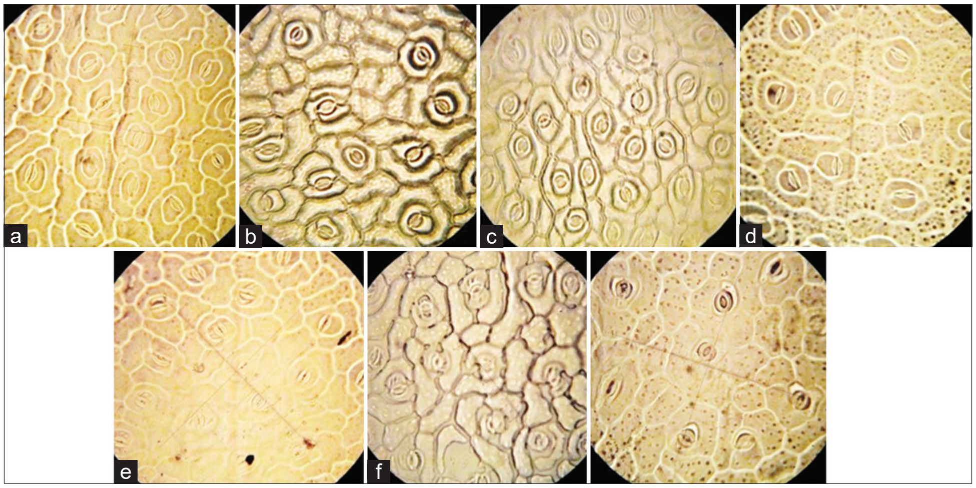

Fig. 1. Lower (abaxial) leaves surfaces of *Vigna sinensis* Savi showing stomata at ×400 for (a) the control, (b) in-target −5 min. (c) in-target −10 min. (d) in-target −20 min. (e) out of target −5 min. (f) out of target −10 min., and (g) out of target −20 min.

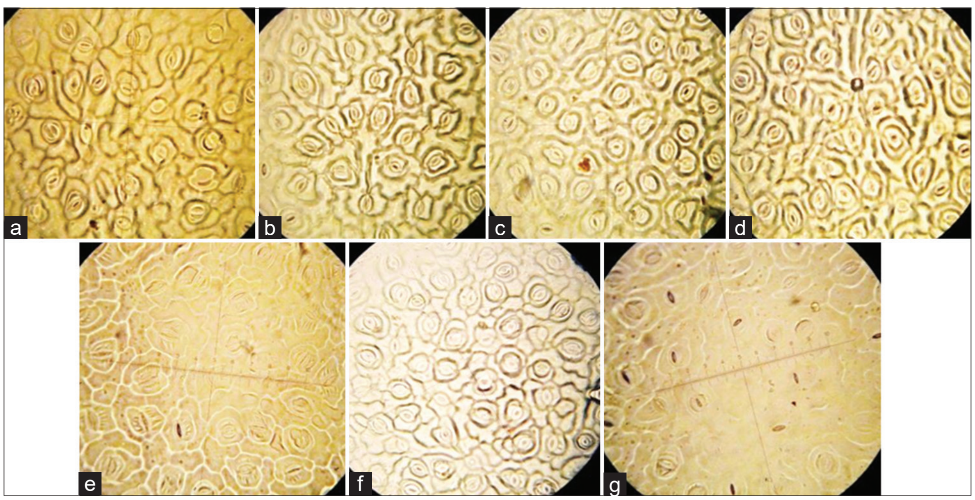

Fig. 2. Upper (adaxial) leaves surfaces of *Vigna sinensis* Savi showing stomata at ×400 for (a) the control, (b) in-target −5 min. (c) in-target −10 min. (d) in-target −20 min. (e) out of target −5 min. (f) out of target −10 min. and (g) out of target −20 min.

micrometer, respectively, in coincides with the treatment 20 min exposure time in the target of radiation source for adaxial leaves surfaces which increased stomata width to 8.67 and 9.67 micrometer, respectively. The present observations showed changes in stomata characteristics under X-ray radiation compared with that not treated. These changes in stomata dimensions under X-ray may due to change in osmotic pressure of epidermal cells which prevent the development of sufficient osmotic pressure in guard cells to open to the same extent as occurs in non-irradiated plants, so the average stomatal opening of X-ray irradiated plants was significantly less compared to non-irradiated plants [13]. Stomatal aperture depends on the genotype of plants and is regulated by many internal and external factors [23].

From Table 3 results, it is shown that exposure seeds to X-ray in target source increases significantly each of stem and leaves dry matter percent to 10.75 and 14.00% compared to that is out of target location (9.13 and 11.88%), respectively, which agrees with Al-Enezi and Al-Khayri [24] whom found an increase in fresh and dry weights of date palm (*Phoenix dactylifera* L.) leaf tissues with increasing the X irradiation dose from 0 to 1500 rad, it also agrees with the results of Arena *et al.* [12] whom found that the high dose of X-rays (50 Gy) increased significantly ($P < 0.001$) leaf dry matter content in faba been young leaves compared to the control leaves. Regarding the time of exposure 5 min exposure to X-ray increased the percent of stem and leaves dry matter content significantly ($P \leq 0.05$) to 13.75 and

TABLE 3: Effects of location and time of exposure cowpea seed to X-radiation, and their interactions in stem and leaf dry matter

Treatments	Stem dry matter (%)	Leaves dry matter (%)
Location of exposure		
In target (L1)	10.75[a]	14.00[a]
Out of target (L2)	9.13[b]	11.88[b]
Time of exposure (min)		
T0	8.50[b]	12.00[bc]
T5	13.75[a]	15.75[a]
T10	9.50[b]	13.50[b]
T20	8.00[b]	10.50[c]
Interactions between location and exposure time		
L1×T0	8.50[cd]	12.00[c]
L1×T5	15.00[a]	17.00[a]
L1×T10	10.00[c]	14.00[bc]
L1×T20	9.50[c]	13.00[bc]
L2×T0	8.50[cd]	12.00[c]
L2×T5	12.50[b]	14.50[b]
L2×T10	9.00[c]	13.00[bc]
L2×T20	6.50[d]	8.00[d]

Means that followed by same letters within column are differ non-significantly at $P \leq 5\%$ according to the Duncan multiple range test

15.75% compared to other treatments, whereas increasing the time of exposure to 20 min decreased the percent of stem and leaves dry matter significantly ($P \leq 0.05$) to 8.00% and 10.5%, respectively. Regarding the interactions between the location and time of exposure, the percent of stem and leaves content of dry matter increased significantly to 15.00% and 17.0% for plants emerged from seeds exposed to X-ray for 5 min on the source target, whereas the lowest values were recorded for seeds exposed to 20 min X-ray out of target. For the X-ray effects, it was seen that the shortest time records led to the highest significant increase, which can be concluded that it likes the effects of low doses of X-radiation which encourage cellular activities and growth whereas higher doses may cause chromosomal abnormalities [25]. Hence, higher X-ray radiation exposure time effect on the growth of plants which reflects on stem and leaves percent of dry matter.

4. CONCLUSIONS

We can conclude that exposing cowpea seeds to X-ray radiation had stimulation effects regarding photosynthesis pigments and stomata characteristics either as increase or decrease responses according to the treatment. It was concluded that the location of the exposure had non-significant effects on photosynthetic pigments, whereas, it effects on stomata characteristics and dry matter content. Best exposure time differ according to the studied characteristics. More studies are recommended about effects of X-ray on wet seeds and seedling by different doses of radiation.

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