

*Full Length Research Paper*

# **Impact of drought on the foliar physiology of maize plants irradiated with gamma radiation**

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**Maize (*Zea mays*), is a plant of economic and nutritional interest, which is often confronted with unfavorable environmental conditions, including water stress. This phenomenon forces the plants to considerably reduce their production by disturbing their metabolism. Seeds of maize of the variety EV8728 were subjected to different doses of gamma radiation (100, 200 and 300 grays) in order to induce mutations in plants that could lead to resistance to water stress. Thus, plants from gamma irradiated seeds were used to evaluate the impact of water stress on leaf physiological parameters (leaf area, density and pore area of stomata and assimilatory pigments). Water stress had a depressive effect on all leaf parameters in maize plants. The degree of sensitivity or tolerance of the plants depends on the dose of irradiation and the intensity of stress applied. The increase in the level of water stress reduces the leaf surface and that of the stomata pores. This in turn increases the density of stomata. In addition, chlorophyll *a* was more sensitive to the effect of water stress than chlorophyll *b*.**

**Key words:** Maize mutants, water stress, gamma irradiation, leaves, stomata ostioles.

## **INTRODUCTION**

Maize (*Zea mays* L.), a tropical annual herbaceous plant of the Poaceae family, is an important cereal crop with more diverse uses than other grains. Maize ranks third after wheat and rice in world cereal production. It is valuable as a food and feed source, not to mention its role as a biofuel (Mohamed et al., 2021). In Côte d'Ivoire, maize has a prominent place in agricultural activities because of its importance in food security. Maize cultivation is present in different regions of the country with an annual national production of 654,738 tons, for a total area of 327,800 ha. Its national consumption is estimated at 28.5 kg per capita per year (Countrystat,

2013). According to Food and Agriculture Organization (FAO) (Anonymous, 2014), production estimates and compared to the situation in neighboring countries, Côte d'Ivoire's maize sector seems to be growing relatively slowly. This observation is justified by the fact that its crop is confronted with numerous biotic (helminthosporiosis, streak, virus, rust, Striga, insects and rodents) and abiotic (water and mineral deficiency, soil degradation) constraints (Lobell et al., 2011; Baffour et al., 2021).

Indeed, drought is a major environmental factor that alters many physiological and metabolic processes in

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plants, ranging from a considerable decrease in growth and development to plant death (Kapoor et al., 2020). In addition, water loss at the plant level leads to an increase in the pressure gradient between the ambient air and the leaf, and consequently an increase in the transpiration rate (Torres-Ruiz et al., 2013). For any plant, drought imposes various physiological and biochemical limits and undesirable effects, of which cell growth is the most affected process (Bouranis et al., 2014; Chen et al., 2015). Thus, under more severe drought conditions, several phenomena such as inhibition of cell division, wall synthesis, proteins, photosynthesis, accumulation of solutes, closure of stomata... are observed (Taiz and Zeiger, 2002; Zulias et al., 2021). Indeed, located abundantly on the lower face, precisely in the epidermis of the leaf, the stomata are responsible for almost all of the exchange of water (stomatal transpiration) and gas between the plant and its external environment (Zhao et al., 2015; Buckley, 2019). Under these stressful conditions, scientific investigations focus on various aspects of plant physiology, such as the response of stomata to drought stress.

This study aim to explore the photosynthetic area in maize variety EV8728 in order to understand not only the variation in leaf area but also the behaviour and mechanism involved by leaf cells under water stress conditions with a view to finding a sustainable solution to the consequences of climate variability.

## MATERIALS AND METHODS

### Study site

The research work was carried out during four months (December 15, 2017 to April 20, 2018) on the research site of the University of Jean Lorougnon Guédé, located in the city of Daloa. The town is located in the region of Haut Sassandra, in the central-western part of Côte d'Ivoire between 6° and 7° North latitude and 7° and 8° West Longitude (Ayolié et al., 2016). The soil substrate is of ferralitic type of medium denatured granitic origin with good agricultural aptitudes and is suitable for all types of crops. The climate is tropical with an average annual temperature of 27.5 °C and an average annual rainfall of between 1000 and 1500 mm per year (Soro et al., 2015).

### Plant material

The biological material consisted of maize seeds of EV8728 variety from the Centre National de Recherche Agronomique (CNRA) of Korhogo/Côte d'Ivoire. These seeds were transported to Seibersdorf in Austria where they were irradiated with gamma rays at doses of 100, 200 and 300 grays.

### Setting up the experiment

The experiment was conducted in a traditional (in vivo) 'greenhouse' 16 m long, 7 m wide and 3 m high. The greenhouse was represented here by a shelter covered with transparent plastic film where the lighting and temperature conditions was close to

natural conditions. Under this greenhouse, 360 pots perforated at the base and then lined with a thin layer of gravel to ensure water and air drainage were used for the experiment. These pots filled with culture substrate (site soil) were treated with nematicides, insecticides and fungicides.

The experimental design was completely randomized with nine treatments (T0×CC, T0×50%CC, T0×25%CC; T1×CC, T1×50%CC, T1×25%CC; T3×CC, T3×50%CC, T3×25%CC) and three replications per treatment. Water supply was the only factor controlled by the experimenter.

### Experimental device

Irradiated and control seeds, disinfected with 10% sodium hypochlorite solution, were first pre-germinated in the dark for 48 h incubation in Petri dishes lined with sterile blotting paper and soaked with sterile distilled water. Then, the pre-germinated seeds were transplanted into the greenhouse pots with one seed per pot. The randomized experimental device was made up of nine elementary blocks, that is three per repetition (100, 50 and 25% of the field capacity). Each elementary block contains 40 pots due to 10 pots in line per irradiation dose (100, 200, 300 grays and control). The distance between repetitions is 60 cm, 40 cm between two elementary blocks, 20 cm between rows and 20 cm between seedlings on the row.

### Determination and application of water stress levels

The different levels of water input in relation to the field capacity (CC) of the soil were determined with a mass of 10 kg of cropland. This mass of normal soil (10 kg) was taken dry for filling the pots represented in the dry weight of the soil, that is P1 (dry weight of the soil). The pots were then watered to saturation, while covering them with aluminium foil to prevent water evaporation. After 48 h of rest, the pots were weighed again, that is P2 (saturation weight). The difference between P2 and P1 was used to determine the field capacity of the soil (CC) contained in the pots according to the following formula :  $CC = (P2 - P1) / P1 \times 100$ .

Three water treatments were selected: treatment 1 (CC), treatment 2 (50% CC) and treatment 3 (25% CC). Eight weeks after transplanting the plants, the different levels of water stress were applied every two days respectively until the maturity of the ears.

### Leaves physiological variables determined

The influence of water stress was evaluated only on the foliar parameters of the different plants (100, 200, 300 grays and control) of maize variety EV8728 under water stress conditions.

### Leaf area

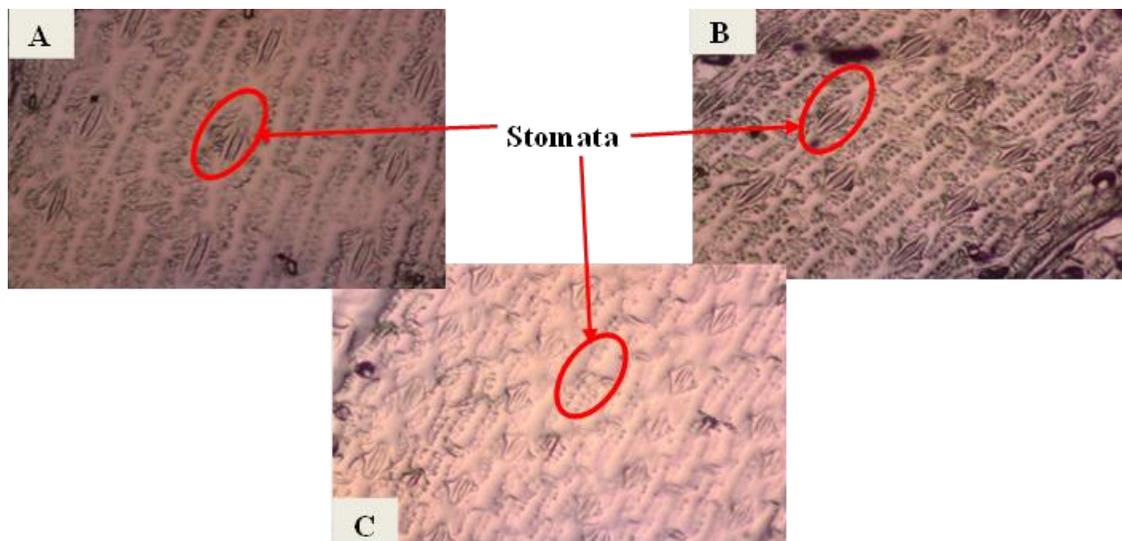
The total leaf area (cm<sup>2</sup>) per plant was determined 45 days after the application of water stress. It consists of measuring the length and median width of the leaves and then deducting the leaf area (LA) by the following formula :

$$SF = (L \times W \times 0.75)$$

SF: Total leaf area per plant, L: length of the leaf, W : median width of the leaf and 0.75: multiplying coefficient.

### Maize leaf stomatal impressions

The stomatal impressions of maize leaves were taken using the



**Figure 1.** Density of stomata at 10x magnification. A: density of leaf stomata of irrigated plants with field capacity (controls); B : density of leaf stomata of irrigated plants with 50 % of field capacity ; C: density of leaf stomata of irrigated plants with 25% of field capacity.

method described by Koffi et al. (2014). The upper surface of the leaf was cleaned with distilled water and drained with blotting paper. Part of this surface was covered with a thin layer of colourless nail polish, while avoiding covering the central vein of the leaf, and left to dry for 15-20 min. After drying, the varnish was meticulously detached with transparent adhesive tape and then glued back onto a glass slide bearing the plant's references. For each corn plant, a leaf was taken at random and three stomata impressions were collected.

#### **Observation and characterization of stomatal imprints**

Once in the laboratory, each of the glass slides was observed with an *Olympus CX31* optical microscope connected to a camera (*LC20*) at x10 magnification to determine the density of the stomata (Figure 1) and at x40 magnification to measure the length and width of the stomata opening in order to calculate the pore surface area of the stomata (Figure 2). Stoma density is defined as the number of stomata per unit leaf area ( $\text{mm}^2$ ) (Djinet et al., 2016). The surface area of stoma pores (SPS) was used to determine the degree of opening of the stomata. This surface was obtained from the formula of Zgallai et al. (2007) :  $\text{SPS} (\text{mm}^2) = (a \cdot b \cdot d) / 4$  with **a** : average length of open stomata in mm ; **b** : average width of open stomata in mm and **d** : number of stomata per  $\text{mm}^2$ .

#### **Extraction and dosage of chlorophyll and carotenoids pigments**

The second leaf of the ear was used for the dosage. Samples were taken after the plant had released all its pollen. The extraction and determination of the chlorophyll and carotenoids pigments were carried out according to the method described by Lichtenthaler (1987). A quantity of 0.1g of leaves cut into small fragments was put into a volume of 10 mL of 95% acetone. The whole was kept in the dark at 4°C for 48 h. A volume of 3 mL of supernatant was then taken for optical density (OD) reading at 663 nm and 647 nm for chlorophylls (Chl *a*, Chl *b* and total Chl) and 470 nm for carotenoids. The calculation of the different concentrations was

performed according to the following formulas:

$$\text{Chl } a (\mu\text{g/mL}) = [12.25 \cdot \text{DO663} - 2.79 \cdot \text{DO647}] \cdot V / 1000m$$

$$\text{Chl } b (\mu\text{g/mL}) = [21.5 \cdot \text{DO647} - 5.10 \cdot \text{DO663}] \cdot V / 1000m$$

$$\text{Total Chl} (\mu\text{g/mL}) = [7.15 \cdot \text{DO663} + 18.71 \cdot \text{DO647}] \cdot V / 1000m$$

$$\text{Car} (\mu\text{g/mL}) = (1000 \cdot \text{DO470} - 1.82 \cdot \text{chl } a - 85.02 \cdot \text{chl } b) \cdot V / (198 \cdot 1000m)$$

Where **V** is the volume (mL) of the crude extract collected after soaking the leaves in acetone and **m** is the mass of the leaves used (g).

#### **Statistical analysis**

The data of the different foliar variables determined in relation to the effect of water stress-radiation on the different irradiated plants of the maize variety EV8728 were processed with the software STATISTICA 7.1. The data were subjected to the two-factor analysis of variance (ANOVA) at the threshold of  $\alpha = 0.05$ . In case of significance, Tukey's HSD test was used to classify the means.

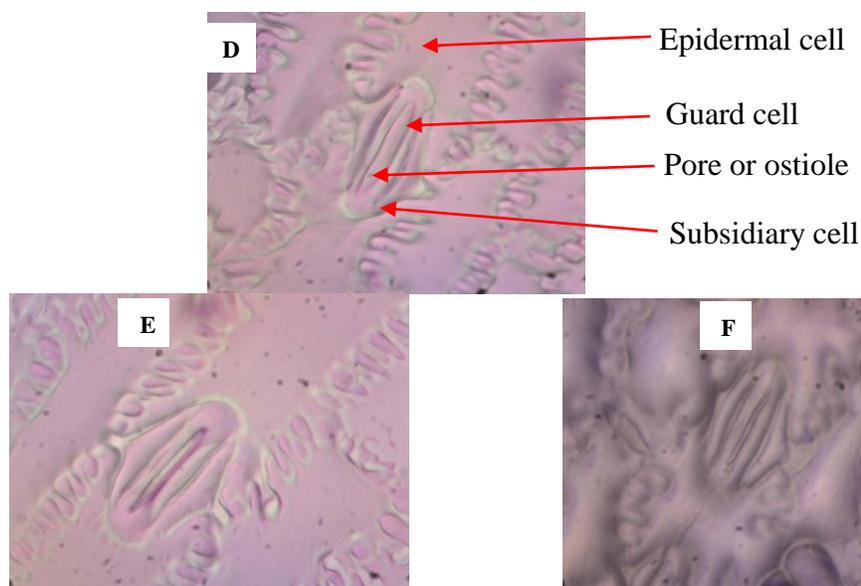
## **RESULTS**

### **Effect of water stress and radiation dose on physiological parameters**

Statistical analysis of the results showed a significant effect of the interaction between water stress and radiation dose on some of the agronomic parameters studied.

#### **Leaf area**

The effect of water stress and irradiation dose reduces



**Figure 2.** Pore area of stomata at 40x magnification. D: stomata of the leaves irrigated plants with the field capacity (control) ; E: stomata of the leaves irrigated plants with 50% of the field capacity ; F: stomata of the leaves irrigated plants with 25% of the field capacity (ostiole) at the target

leaf area in general, in the maize plants studied (Table 1). The combination of water stress and irradiation dose resulted in a considerable reduction of the average leaf area from 8239.87 cm<sup>2</sup> to 2690 cm<sup>2</sup> respectively for the control treatment (CCxD0) and the 50%CCxD300 treatment. However, for each amount of water supplied, plants from seeds irradiated at the D100 dose (100Gy) have the highest mean leaf area (10577.03cm<sup>2</sup> ; 7221.23 cm<sup>2</sup> and 6054.75 cm<sup>2</sup>) while those from D300 (300Gy) have the lowest mean leaf area (5045.39 cm<sup>2</sup> ; 4075.09 cm<sup>2</sup> and 2690 cm<sup>2</sup>). On the other hand, D0 (unirradiated) and D200 (200Gy) have an average leaf area intermediate between D100 and D300. Thus, as the severity of the stress increases, the leaf area is reduced. Statistical analysis of the data confirmed a significant effect ( $P= 0.046$ ) for water stress level and irradiation dose on the expression of leaf area of the maize variety EV8728.

In addition, Tukey's HSD test at the 5% threshold identified nine treatment groups: CCxD100 (1st group) followed by CCxD0 (2nd group); 50%CCxD100 and CCxD200 (3rd group); 25%CCxD100 and 50%CCxD0 (4th group); CCxD300 and 25%CCxD0 (5th group); 50%CCxD200 (6th group); 25%CCxD200 (7th group); 50%CCxD300 (8th group) and finally 25%CCxD300 (9th group).

### Density of maize leaf stomata

Gamma irradiation and water stress induce a general

increase in the average density of the stomata (Table 1). The 25% CC treatment (stress) induced more stomata in the leaves regardless of irradiation dose. However, the plants from 300 grays irradiated seeds recorded the highest stomata density. This increase ranges from 1050 stomates/mm<sup>2</sup> for the control treatment (CCxD0) to 1950 stomates/mm<sup>2</sup> for the 50%CCxD300 treatment. Nevertheless, D0 and D100 have lower average stomatal densities for each irrigation level than those in D200 and D300, which have higher average stomatal densities. So, analysis of variance of the data is reveal a significant difference ( $P = 0.002$ ) between the mean stomatal densities of maize leaves at the 5% threshold. Tukey's HSD test at the 5% threshold identified seven groups: 25%CCxD200 and 25%CC D300 (1st group) followed by 25%CCxD100 (2nd group); 25%CCxD0 and 50%CCxD300 (3rd group); 50%CCxD200 (4th group); 50%CCxD100, 50%CCxD0 and CCxD300 (5th group); CCxD200 (6th group) ;CC\*D100 and CCxD0 (7th group).

### Pores area of the stomata (ostioles) of maize leaves

The mean of stomata pore area (ostioles) of the maize leaves studied under the influence of irradiation and water stress are shown in Table 1. The pore area of the stomata are reduced under the influence of irradiation and water stress.

The reduction in the mean pore area of the stomata ranges from 0.027 mm<sup>2</sup> in the control treatment (CCxD0) to 0.005 mm<sup>2</sup> in the 25%CC\*D300 treatment. For each

**Table 1.** Influence of water stress/ irradiation interaction on leaf area, stoma density and stoma area of greenhouse maize plants.

Treatment	Irradiation dose (gry)	Leaf area (cm <sup>2</sup> )	Density of stomata (stomata/mm <sup>2</sup> )	Pore area of stomata(mm <sup>2</sup> /stomata)
<b>CC*D0 (control)</b>	D0 (control)	8239.87 ± 647 <sup>b</sup>	1050 ± 65 <sup>f</sup>	0.027 ± 0.004 <sup>a</sup>
	100	10577.03 ± 736 <sup>a</sup>	1089 ± 43 <sup>f</sup>	0.022 ± 0.002 <sup>b</sup>
	200	7107.02 ± 265 <sup>bd</sup>	1174 ± 91 <sup>ef</sup>	0.020 ± 0.002 <sup>bc</sup>
	300	5045.39 ± 826 <sup>ef</sup>	1225 ± 100 <sup>e</sup>	0.022 ± 0.003 <sup>b</sup>
<b>50%CC</b>	D0 ( control )	6011.77 ± 597 <sup>de</sup>	1239 ± 111 <sup>e</sup>	0.015 ± 0.003 <sup>cd</sup>
	100	7221.23 ± 500 <sup>bd</sup>	1238 ± 118 <sup>e</sup>	0.018 ± 0.002 <sup>c</sup>
	200	5432.63 ± 364 <sup>f</sup>	1520 ± 71 <sup>c<sup>d</sup></sup>	0.017 ± 0.003 <sup>c</sup>
	300	4075.09 ± 397 <sup>g</sup>	1689 ± 190 <sup>bc</sup>	0.013 ± 0.0002 <sup>d</sup>
<b>25%CC</b>	D0 ( control )	5182.24 ± 72 <sup>ef</sup>	1671 ± 124 <sup>bc</sup>	0.010 ± 0.001 <sup>e</sup>
	100	6054.75 ± 425 <sup>de</sup>	1723 ± 70 <sup>b</sup>	0.012 ± 0.003 <sup>d</sup>
	200	4329.19 ± 212 <sup>f<sup>g</sup></sup>	1915 ± 176 <sup>a</sup>	0.007 ± 0.001 <sup>f</sup>
	300	2690.00 ± 760 <sup>h</sup>	1950 ± 81 <sup>a</sup>	0.005 ± 0.001 <sup>f<sup>g</sup></sup>
<b>P</b>		<b>0.046</b>	<b>0.002</b>	<b>0.015</b>
<b>F</b>		<b>2.409</b>	<b>9.015</b>	<b>3.061</b>

P = Approximate probability of the Tests ; F = Fischer's constancy. Mean values are followed by their standard deviation (±). Values with the same letters are not significantly different (Tukey's 5% test). CCxD0: 100% field capacity - non-irradiated; CCxD100: 100% field capacity - 100 Gy dose; CCxD200: 100% field capacity - 200 Gy dose; CCxD300: 100% field capacity - 300 Gy dose; 50%CCxD0: 50% field capacity - non-irradiated; 50%CCxD100: 50% field capacity - 100 Gy dose; 50%CCxD200 : 50% field capacity - 200 Gy dose; 50%CCxD300: 50% field capacity - 300 Gy dose; 25%CCxD0: 25% field capacity - non-irradiated; 25%CCxD100: 25% field capacity - 100 Gy dose; 25%CCxD200: 25% field capacity - 200 Gy dose; 25%CCxD300: 25% field capacity - 300 Gy dose.

level of stress, the mean stomatal surface area is highest in D100 (0.018 mm<sup>2</sup> and 0.12 mm<sup>2</sup>) and lowest in D300 (0.013 mm<sup>2</sup> and 0.005 mm<sup>2</sup>). This reduction in the mean pore surface area of stomata in corn variety EV8728 is significant (P = 0.015).

Tukey's HSD test at the 5% threshold identified nine water treatment level groups : CCxD0 (1st group) followed by CCxD100 and CCxD300 (2nd group); CCxD200 (3rd group); 50%CCxD100 and 50%CCxD200 (4th group); 50%CCxD0 (5th group); 50%CCxD300 and 25%CCxD100 (6th group); 25%CCxD0 (7th group); 50%CCxD200 (8th group) and finally 25%CCxD300 (9th group). The stomata of the leaves have variable opening areas.

## Foliar pigments of maize plants

### Chlorophyll pigments

In general, water stress and irradiation have an inhibitory effect on the synthesis of chlorophyll pigments (Table 2). The average chlorophyll content (a) is four times higher than chlorophyll (b) in control. This indicates more chlorophyll (a) than chlorophyll (b) in the leaves of the maize variety EV8728 studied. This average chlorophyll (a) and (b) content decreases as the water deficit in the growing medium increases. Indeed, with a small amount of water supplied, the average chlorophyll (a), (b) and total chlorophyll content decrease considerably. Thus, the reduction in content goes

from 1.304µg/ml (CC\*D0) to 0.44µg/ml (25%CC\*D300) for chlorophyll (a), from 0.318 µg/ml (CC\*D0) to 0.16 µg/ml (25%CC\*D300) for chlorophyll (b) and from 1.612 µg/ml to 0.616µg/ml for total chlorophyll. For each stress level, the average chlorophyll (a), (b) and total chlorophyll content are higher at D100 and lower at dose D300. This reduction is significant (p = 0.015) for chlorophyll (b) but not for chlorophyll (a) (p = 0.72) and total chlorophyll (p = 0.42). Comparison of the means for chlorophyll (b) with Tukey's HSD test at the 5% cut-off indicates eight distinct treatment groups: the 1st group is represented by CCxD0; the 2nd group by CCxD100; the 3rd group by CCxD200; the 4th group by 50% CC x D100; the 5th group by

**Table 2.** Influence of Water Stress / Irradiation Interaction on Leaf Pigments in Greenhouse maize Plants.

Water stress level	Irradiation dose (gry)	Chlorophyll a ( $\mu\text{g} / \text{ml}$ )	Chlorophyll b ( $\mu\text{g} / \text{ml}$ )	Total chlorophyll ( $\mu\text{g} / \text{ml}$ )	Caroténoid ( $\mu\text{g} / \text{ml}$ )
<b>CC</b>	D0 (control)	1.304 $\pm$ 0.15 <sup>a</sup>	0.318 $\pm$ 0.004 <sup>a</sup>	1.612 $\pm$ 0.149 <sup>a</sup>	0.856 $\pm$ 0.117 <sup>a</sup>
	100	1.281 $\pm$ 0.13 <sup>a</sup>	0.280 $\pm$ 0.002 <sup>ab</sup>	1.561 $\pm$ 0.111 <sup>a</sup>	0.849 $\pm$ 0.034 <sup>a</sup>
	200	1.238 $\pm$ 0.10 <sup>a</sup>	0.241 $\pm$ 0.002 <sup>b</sup>	1.479 $\pm$ 0.094 <sup>b</sup>	0.806 $\pm$ 0.186 <sup>b</sup>
	300	1.018 $\pm$ 0.11 <sup>b</sup>	0.214 $\pm$ 0.003 <sup>ab</sup>	1.228 $\pm$ 0.107 <sup>c</sup>	0.820 $\pm$ 0.075 <sup>b</sup>
<b>50 % CC</b>	D0 (control)	0.673 $\pm$ 0.13 <sup>de</sup>	0.208 $\pm$ 0.003 <sup>c</sup>	0.882 $\pm$ 0.148 <sup>e</sup>	0.458 $\pm$ 0.020 <sup>de</sup>
	100	0.871 $\pm$ 0.07 <sup>c</sup>	0.23 $\pm$ 0.002 <sup>bc</sup>	1.111 $\pm$ 0.082 <sup>cd</sup>	0.641 $\pm$ 0.018 <sup>c</sup>
	200	0.840 $\pm$ 0.03 <sup>c</sup>	0.20 $\pm$ 0.003 <sup>bc</sup>	1.04 $\pm$ 0.317 <sup>d</sup>	0.570 $\pm$ 0.071 <sup>cd</sup>
	300	0.720 $\pm$ 0.07 <sup>d</sup>	0.19 $\pm$ 0.002 <sup>cd</sup>	0.898 $\pm$ 0.376 <sup>e</sup>	0.518 $\pm$ 0.080 <sup>d</sup>
<b>25 % CC</b>	D0 (control)	0.470 $\pm$ 0.05 <sup>f</sup>	0.16 $\pm$ 0.001 <sup>de</sup>	0.620 $\pm$ 0.051 <sup>g</sup>	0.294 $\pm$ 0.016 <sup>g</sup>
	100	0.55 $\pm$ 0.05 <sup>e</sup>	0.19 $\pm$ 0.003 <sup>cd</sup>	0.730 $\pm$ 0.033 <sup>f</sup>	0.412 $\pm$ 0.022 <sup>e</sup>
	200	0.52 $\pm$ 0.01 <sup>e</sup>	0.17 $\pm$ 0.001 <sup>e</sup>	0.685 $\pm$ 0.011 <sup>fg</sup>	0.381 $\pm$ 0.030 <sup>ef</sup>
	300	0.44 $\pm$ 0.05 <sup>f</sup>	0.16 $\pm$ 0.001 <sup>de</sup>	0.616 $\pm$ 0.045 <sup>g</sup>	0.329 $\pm$ 0.031 <sup>f</sup>
<b>P</b>		<b>0.001</b>	<b>0.015</b>	<b>0.01</b>	<b>0.001</b>
<b>F</b>		<b>10.08</b>	<b>3.061</b>	<b>6.025</b>	<b>2.039</b>

P = Approximate probability of the Tests ; F = Fischer's constancy. Mean values are followed by their standard deviation ( $\pm$ ). Values with the same letters are not significantly different (Tukey's 5% test). CC\*D0: 100% field capacity - non-irradiated; CC\*D100: 100% field capacity - 100 Gy dose; CC\*D200: 100% field capacity - 200 Gy dose; CC\*D300: 100% field capacity - 300 Gy dose; 50%CC\*D0: 50% field capacity - non-irradiated; 50%CC\*D100: 50% field capacity - 100 Gy dose; 50%CC\*D200 : 50% field capacity - 200 Gy dose; 50%CC\*D300: 50% field capacity - 300 Gy dose; 25%CC\*D0: 25% field capacity - non-irradiated; 25%CC\*D100: 25% field capacity - 100 Gy dose; 25%CC\*D200: 25% field capacity - 200 Gy dose; 25%CC\*D300: 25% field capacity - 300 Gy dose.

CCxD300, 50%CC\*D0, 50%CCxD200 and 50%CCxD300; the 6th group by 25%CCxD100 and 25%CCxD200; the 7th group by 25%CCxD300 and the 8th group by 25%CCxD0.

### Carotenoids

As with all chlorophyll pigments dosed in this work, the very high average leaf carotenoid content in the controls is reduced with water stress and irradiation dose severity. Values range from 0.856  $\mu\text{g}/\text{ml}$  (CC\*D0) to 0.234  $\mu\text{g}/\text{ml}$  (25%CC\*D0). The D100 dose has the highest

average carotenoid content for the different levels of water stress. However, statistical analysis of the data reveal a significant difference ( $P = 0.001$ ) between the mean carotenoid content of corn leaves at the 5% threshold (Table 2).

### DISCUSSION

The results relating to the different physiological variables of the leaves of corn plants of the EV8728 variety obtained from irradiated seeds were influenced by water stress and irradiation. The corollary of water stress was the inhibition of

leaf expansion. Indeed, the lack of water has led to a significant reduction in the photosynthetic surface area (leaves). The leaf area of maize plants receiving severe water deficit was much smaller compared to the control treatment (CC xD0). The reduction is more marked at the highest level of water stress, at 25% of the field capacity (25% CC). These results agree with the work of Lauer (2005), on the behavior of corn in dry weather. This author has shown that the application of water stress during vegetative development considerably reduces the leaf area. Similar results were obtained by Attia (2007) on cotton, Ayolié et al. (2016a) on tomato, Ved and

Sukhbir (2020) in market gardeners and Ved et al. (2021) in cucumber cultivars. According to Durand et al. (1997), It has been shown that the expansion of aerial organs immediately and sharply decreases with water deficit in all plants, including those known to be resistant.

Kramer and Boyer (1995) and Lebon (2006) have shown that the decrease in leaf area under a severe water regime is an adaptive mechanism in plants aimed at limiting leaf transpiration when water conditions become unfavorable. Regarding the irradiation doses, the results showed that the gamma ray irradiation at a dose of 100 Gy has a stimulating effect on the leaf surface. While high doses of gamma irradiation (200 Gy and 300 Gy) have a depressive effect on the expression of the photosynthetic surface. Similar results have been reported by Kim et al. (2005) on red pepper. The effects of low dose gamma irradiation are said to stimulate growth by altering the hormonal signaling network in plant cells via improving the antioxidant capacity of cells to simply overcome daily stressors such as variations in intensity. Light, soil moisture and temperature in the growing medium (Kim et al., 2004). The determination of the surface of the stomatal pores (ostioles), at the level of the upper surface of the corn leaves of the different levels of water stress, made it possible to demonstrate the reduction of these compared to the control. Indeed, the severity of the water stress leads to the reduction of the surface of the ostioles. Their results agree with those of Zgallai et al. (2007) on tomato and Lawson et al. (2014) on Arabidopsis. Stomatal closure occurs when two guard cells surrounding the stomatal opening lose turgor pressure and close the opening. There are many signals that induce stomatal closure, among which the most well-known signal is probably abscisic acid (ABA). There are several secondary messengers, such as  $Ca_2^+$ ,  $H_2O_2$  and nitrous oxide (NO) that contribute to stomatal closure (Manzoni et al., 2011; Arve et al., 2013). Passive loss of turgor pressure also results in stomatal closure (Arve et al., 2011). According to Pantin et al. (2013) ABA promotes stomatal closure in two ways: i) via its biochemical effect on guard cells. Abscisic acid (ABA) produced in roots and leaves during water stress is transported to guard cells by ATP binding cassette (ABC) transporters and activates signaling pathways leading to stomatal closure, ii) an indirect water influence. This action occurs by reducing the water permeability in the vascular tissues of the leaf. The reactivity of leaf water conductance to ABA varies among species and could provide a physiological basis for isohydric or anisohydric behavior in plants. The chlorophyll pigment content of corn leaves is negatively affected by the lack of water in the soil. The chlorophyll (b) content decreases significantly with the level of water stress applied. The reduction in the chlorophyll content (b) is more marked with the treatment of 25% of the field capacity (CC). On the other hand, that of the carotenoid does not present any difference. Our results are in agreement with the work of Beniken et al. (2013) on clementine, Ayolié et al.

(2016a) on tomato, Gizie et al. (2021) on wheat, Laura and Davide (2021) on Vitis hybrids. According to the latter authors, the reduction or increase in the content of photosynthetic pigments depends on the adaptability or sensitivity of the varieties studied. The work of Noreen et al. (2020) on corn, in agreement with the authors, showed that the carotenoid content does not vary according to the varieties studied under conditions of salt stress. On the other hand, that of total chlorophylls was significant. According to Impes (1989), the reduction in the content of leaf pigment in stressed plants is explained by the fact that these leaf pigments are much degraded during stress while in unstressed plants, these pigments are more and more synthesized. Moreover, according to Bousba et al. (2009), the fall in the pigment content is the consequence of the reduction in the opening of the stomata aimed at limiting water loss through transpiration and by increasing the resistance to the entry of atmospheric  $CO_2$  necessary for photosynthesis.

## Conclusion

The results obtained, at the end of the study on the effect of water stress / irradiation interaction on the leaves physiological variables studies of the maize variety EV8728 in central-western Côte d'Ivoire, led to the following conclusions. Indeed, this study revealed that the effect of the hydric deficit was well marked between the control plants and those stressed during the experiment. Water stress had a depressing effect on maize plants on all foliar variables studies. The leaf expansion, leaf pigments accumulation and stomata density depending on seed irradiation dose and the stress intensity applied. The increase in the level of hydric stress reduces the foliar area and the area of the stomatal pores. This in turn leads to an increase in the density of the stomata, which are organs that regulate transpiration, and therefore exchange between the plant and the atmosphere. Chlorophyll *a*, chlorophyll *b* and total chlorophyll levels are very sensitive parameters that represent indicators of the degree of tolerance of the maize variety (EV8728). On the other hand, chlorophyll *a* was more sensitive to the effect of water stress than chlorophyll *b*.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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