

Variation of some biochemical markers of stress in six (06) chilli cultivars (*Capsicum* spp) under water deficit conditions at the flowering and fruiting stages of their development

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In Togo, pockets of drought at unexpected periods in crop cycles constitute a major obstacle for the peppers cultivation. The study aims to assess water deficit tolerance of six (06) chilli cultivars which differ by their physiology and their importance value index, at the flowering and fruiting stage of their development. The test was carried out in 10 L vegetation pots in a greenhouse using an experimental split-plot device. The plants are irrigated by successive weighing of the pots, at a period of 3 days, during which the controls are irrigated at 70 % of the useful water reserve (UWR), while the stressed treatments maintain restriction of water content to 30 % of the UWR. At the end of the water shortage cycle, proline, total chlorophylls and malondialdehyde were assayed by spectrophotometry. The results show a better level of tolerance of the cultivars *Gobi*, *Tongor*, ICRAD-I and ICRAD-III with a strong accumulation of proline and weak malondialdehyde, as well as a weak degradation of chlorophyll pigments, unlike the cultivars *Adibolo* and *Gboyébéssé*. These results are useful for effective crop monitoring of chilli and for a better planning of an irrigation program.

Keywords: Chili; drought; tolerance; biochemical markers; Togo.

INTRODUCTION

Chili (*Capsicum* spp) is a well-known and widely used vegetable in the world originated from South and Central America (Adetula and Olakojo, 2006). It is used as medicine for certain diseases treatment, as well as spice for meals and especially sauces. In Togo, pepper production in 2012 amounted to approximately 1,931 tons (DSID, 2013) with prices between from 95 to 1,300 FCFA per kilogram (for fresh peppers) and from 1,200 to 6,000 FCFA per kilogram (for dried peppers). However, threats related to climate variability and environmental degradation are likely to affect its culture and yield.

Indeed, Togo is facing since several years serious changes in the rainfall regime that result to long-term droughts at unexpected periods (Lemou, 2008; Adewi et al., 2010). During 1961 to 2010 period, strong thermal growth was recorded in the southern (1.0 and 1.6 ° C) and northern (0.9 and 1.6 ° C) plains of Togo. In these regions, maximum extreme temperatures occur almost every year and can reach up to 40° C. Average rainfall between 1986 and 2005 decreased by 89 ± 22 mm across the country. In the guinean climatic zone, an average of 27 days separate the early dates from the late dates for the start of the short rainy season which may almost not exist in this part of the country. The announced climate change will worsen this situation in the future. In addition to variability and poor rainfall distribution relative to cropping cycles, global warming will lead to increased water losses through evapotranspiration (IPCC, 2007). Among all the bioclimatic factors

that control the functioning of ecosystems, water deficit is a component whose modifications must be foreseen and its consequences quantified.

Drought significantly affects plant growth. It is undoubtedly one of the major factors limiting yield under natural conditions (Ciais et al., 2003; Webber et al., 2018). The plasticity of plants allows the development of adaptive responses to the hydric state of the soil which fluctuates regularly and in a little predictable manner according to the circadian and seasonal cycles. Each water state's deviation from its optimum does not necessarily result in stress. Stress is the alteration of the physiological condition caused by factors that tend to change its balance (Chaves et al., 2003; Hu et al., 2006).

Thus, the need to increase yields and improve the quality of peppers in Togo, in climate change context led to this study. It aims to assess the effect of a water deficit on the variation of some biochemical markers including the synthesis of proline and malondialdehyde, as well as chlorophyll pigments degradation in six (06) chilli cultivars in Togo, in order to identify the most tolerant to an induced stress. These results could be used to define a better irrigation policy for chilli crops, as well as for grafting or varietal creation approaches.

MATERIAL AND METHODS

Study framework and plant material

The test took place at the Agronomic Experimentation Station (SEA) (6° 10'N, 1° 12'E; altitude = 50 m, slope <1 %) at University of Lomé - High Agronomic School (ESA), between August 2019 and January 2020. Six (06) chilli cultivars including four (04) locals (Gboyébéssé, Gobi, Adibolo, Tongor) and two (02) imported (ICRAD -I and ICRAD-III) are used for the tests (Figure 1 and Table 1). The cultivars selected are, according to peasant perception, the best adapted to the climate, the most tolerant to diseases and the most adopted on the market (high index value).

Experimental set up and irrigation techniques

The test is carried out in a greenhouse, using 10 L vegetation pots (25 cm deep, 25 cm above diameter and 16 cm below diameter). Each pot is filled with 7 kg of substrate made up of sieved potting soil (2 mm), sterilized and enriched with 1/10 compost. Because pepper grows better on well-drained, non-hydromorphic soils, the bottom of the pots were drilled with four (04) holes to let the water drain after watering. This procedure is a perfect simulation of crop progressive water stress in open field, where it's often difficult to achieve real drought situations (Bravdo, 2005). The chosen experimental set up is a split-plot with three (03) repetitions (Figure II) with two (02) interacting factors: the varietal type and the water regime. The experimental unit consists of three (03) pots. The induction of the water deficit consisted in a drop in irrigation, from 70 % of the Useful Water Reserve (UWR) for the control to 30 % of the UWR (stressed) at the flowering and fruiting stages, two (02) stages tasked as being most sensitive to drought in chili peppers development (Jaimez et al., 2000). The induced water stress lasted 21 days, corresponding to the average duration of the drought's pockets recorded during the farming cycles in Togo (DNM, 2007). At the end of this cycle of water shortage, irrigation is resumed as for the control (70 % of the UWR).

Plants are irrigated by a successive weighing of the pots with a periodicity of three (03) days during which the control is supplied in water at 70 % of the UWR, while the treatments are supplied in water at 30 % of the UWR (Bokobana, 2017). Indeed, considering that all the water present in the soil between the wilting point and the field capacity is the useful water reserve (UWR) and that only 2/3 of this water is easily usable by the plant (Musy and Soutter, 1991; Gate, 1995), it is determined that below the threshold of 33.33 % (1/3 of the UWR), the plant will face difficulties in removing water from the soil. It will therefore undergo, water stress. The UWR is calculated according to the

following formula (Baize, 2000; Bokobana et al., 2019):

h: samples number ; Θ_{fc} 2,7: humidity at field capacity in %, Θ_{wp} 4,2: humidity at permanent wilting point in %, % Tfine: percentage in fine particles, E: soil depth in dm, Da: Soil bulk density.

The field capacity (Θ_{fc} 2.5) and the permanent wilting point (Θ_{wp} 4.2) of the substrate were determined at the Saria soil physics laboratory in Burkina-Faso. The substrate filled in the pots is watered daily at field capacity for a week before transplanting. This preparation of the substrate avoids any compaction which would impede root exploration and lead to the poor development of the plant.

The seeding is carried out in nursery to strengthen the root system and obtain vigorous plants that will be transplanted into the pots. For a better growing in pots, only vigorous, short and stocky seedlings about 10 to 15 cm height, with 6 to 8 leaves without any remarkable disease symptoms are transplanted at a rate of one per pot. The environmental conditions in the greenhouse during the test are as follows: 12 h photoperiod, average temperatures of 26°C / 29°C / 27°C at 8 h / 14 h / 17 h and relative air average humidity of 81 % / 66 % / 74 %.



Figure 1: Tested cultivars: a. Tongor; b- Gboyébésé; c- Gobi, d- ICRAD I, e- Adibolo, f- ICRAD III

Cultivars	Related species	Disease Resistance	Total capsaicin content (mg/100 g of fresh material)	Cycle	Yield
Gobi	<i>C. frutescens</i>	N/A	N/A	N/A	N/A
Tongor	<i>C. chinense</i>	N/A	N/A	N/A	N/A
Adibolo	<i>C. annuum</i>	N/A	N/A	Court	N/A
Gboyébessé	<i>C. chinense</i>	N/A	N/A	<u>nd</u>	N/A
ICRAD-I	<i>C. annuum</i>	PVY (R), BW (R), Anthr (R)*	15.3*	75 à 90 J*	15 t.ha ⁻¹ *
ICRAD-III	<i>C. annuum</i>	CMV (R), PVY (R), Large fruit check*	16.6*	75 à 90 J*	30 t.ha ⁻¹ *

CMV: Cucumber Mosaic Virus (R: resistant); PVY: Potato virus Y (R: resistant); BW: Bacterial Wilt (R: resistant), * (ITRA, 2015).

Table 1: Tested varieties profiles

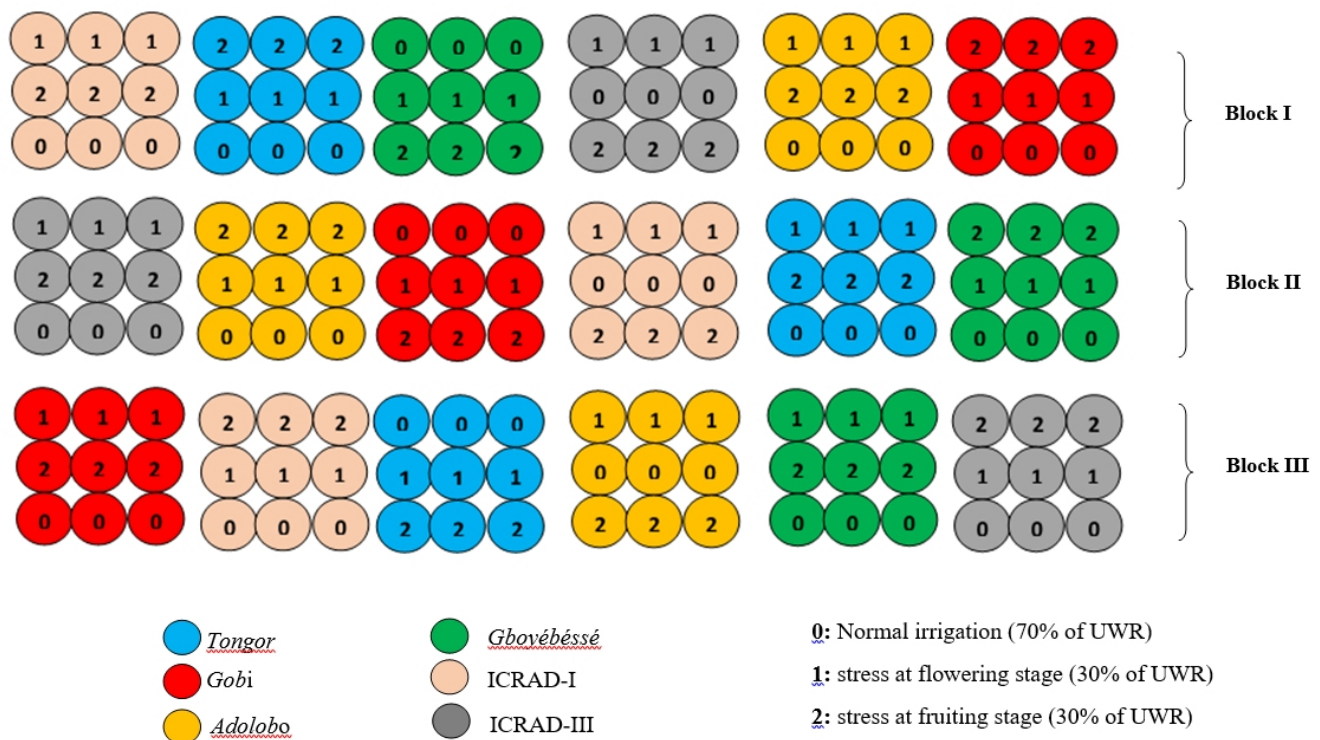


Figure 2: Plants disposition for experimental water deficit induction (Split Plot 3 x 4)

Foliar proline assay

Bogdanov method adapted to the leaves (Bogdanov, 1999) was used. It consists in measuring the absorbance at 510 nm of a leaf extract (25 mg / mL of water) and of a standard proline solution (32 µg / mL). Thus, in 5 mL test tubes is introduced 0.5 mL of leaf extract or standard proline solution, or distilled water (for the "blank"). 1 mL of formic acid (100 %) and 1 mL of ethylene glycol (3 %) are then added. The closed tubes are vigorously agitated for 15 minutes at room temperature and then placed for 15 minutes in a boiling bath. After adding 2.5 mL of 50 % 2-propanol, the tubes are

placed in a water bath at 70°C for 10 minutes, then replaced at room temperature. 45 minutes later, the absorbance at 510 nm of the tubes contents is read with a spectrophotometer. The proline content of the leaves, estimated in $\mu\text{g.mg}^{-1}$ of proteins, is then determined by the following formula:

$$\text{TP} = (\text{Ae} / \text{As} \times \text{Ms} / \text{Mf}) / \text{Qp}$$

Ae: Absorbance of the leaf extract; As: Absorbance of the standard proline solution; Ms: Mass of proline in the standard solution (μg); Mf: Fresh leaf mass (g); Qp: Amount of protein (mg.g^{-1} fresh matter).

Determination of total chlorophyll pigments

To assess the functional integrity of photosystem II (PS II) to water stress, total chlorophyll pigments has been assessed. The total chlorophyll pigments are extracted by solubilization in acetone 80 % (Queval et al., 2007). 1.2 mL of 80 % acetone are thus added to the 50 μL of leaf grind contained in an Eppendorf tube. After standing for 3 hours in the dark (in the refrigerator), centrifugation at 14,000 rpm for 10 minutes is carried out. The supernatant then collected was used to read the absorbances at 645 nm and 663 nm (visible wavelength) using 80% acetone for the solution of the "blank". Total chlorophyll concentration expressed in mg.g^{-1} of fresh matter is determined by the formula:

$$\text{Chl.T} = [(8.02 \times \text{A663}) + (20.2 \times \text{A645})] / \text{MF}$$

CT: Total chlorophyll (mg.g^{-1} fresh matter); A663 and A645: absorbances read at 663nm and 645nm respectively; MF: Fresh mass of the leaf.

Determination of leaf malondialdehyde (MDA)

MDA is one of the final products of peroxidation of membrane lipids (Lacan and Baccou, 1998; Guichardant et al., 1994). This peroxidation is the symptom most associated with oxidative damage. It is often used as an indicator of oxidative stress (Zhang and Kirkham, 1994). The MDA assay is therefore an effective mean to assess the damage of oxidative stress on the membrane (Katsuhara et al., 2005). The MDA is assayed according to the method of Heath and Paker (1968), on 250 mg of fresh plant material removed and ground. The ground material is suspended in 5 mL of trichloroacetic acid (5 % w/v) containing 1.25 % glycerol. The homogenate is centrifuged at 12,000 rpm for 10 min and filtered through Whatman N°1 paper. The supernatant is collected in test tubes. To 2 mL of supernatant are added 2 mL of

0.67 % thiobarbituric acid (prepared in distilled water). The whole mixed with the vortex is heated for 30 min in a water bath at 100°C, then cooled in ice and finally centrifuged for one minute. The absorbance is read at 532 nm and then at 600 nm. The optical density is then corrected by subtracting the non-specific absorbance at 600 nm. The amount of MDA is calculated using a molar extinction coefficient of $155 \text{ mM}^{-1}.\text{cm}^{-1}$, according to the Beer-Lambert law:

$$\text{Absorbance} = \epsilon \times L \times [C]$$

ϵ : molar extinction coefficient; L: width of the tank (1cm); [C]: Concentration in MDA (mg.g^{-1} fresh matter).

RESULTS

Water deficit effects on biochemical variables

Analysis of variance

The analysis of variances (Tables 2 and 3) shows that the water deficit significantly influenced the biochemical measurements in chilli plants, regardless the cultivar.

Source	df	Flowering stage								
		MDA			Prol			Chl		
		AS	P	CM	P	AS	P	AS	P	
		F	F	F	F	F	F	F	F	
Wr	1	41,07	***	28,81	***	10,55	***	109,22	273,16	
Cultivars	5	21,78	*	02,39	ns	05,09	*	05,41	207,41	
Wr*Cultivars	11	53,37	**	53,71	**	8,82	**	08,65	317,27	
				20,10						

df: degree of freedom; AS: average square; Wr: water regime; F: Fisher factor; * significant at the 5% threshold; ** significant at the 1% threshold; *** significant at the 0.1% level; ns = non-significant. MDA: malondialdehyde; Prol: proline; Chl: total chlorophyll.

Table 2: Variance analysis of biochemical variables at the flowering stage

Source	df	Fruiting stage								
		MDA			Prol			Chl		
		AS	P	AS	P	AS	P	AS	P	
		F	F	F	F	F	F	F	F	
Wr	1	11,59	***	23,10	***	09,54	***	371,81	216,25	
Cultivars	5	05,66	**	04,20	ns	05,12	*	13,32	145,56	
Wr*Cultivars	11	09,50	***	06,68	**	49,22	**	151,18	218,45	
				55,47						

df: degree of freedom; AS: average square; Wr: water regime; F: Fisher factor; * significant at the 5% threshold; ** significant at the 1% threshold; *** significant at the 0.1% level; ns = non-significant. MDA: malondialdehyde; Prol: proline; Chl: total chlorophyll.

Table 3: Variance analysis of biochemical variables at the fruiting stage

Influence of water deficit on the proline content in leaves

The synthesis of proline increased significantly with the application of water deficit regardless of the cultivar (Table 4).

Water regime	Cultivar	Proline content ($\mu\text{g}\cdot\text{mg}^{-1}$ of protein)		Chlorophyll content ($\text{mg}\cdot\text{g}^{-1}\text{mf}$)		Malondialdehyde content ($\text{mg}\cdot\text{g}^{-1}\text{mf}$)	
		Flowering stage	Fruiting stage	Flowering stage	Fruiting stage	Flowering stage	Fruiting stage
Normal	<i>Adibolo</i>	01,62 \pm 0,26 ^d	02,52 \pm 0,33 ^c	02,03 \pm 0,32 ^a	02,62 \pm 0,14 ^b	05,16 \pm 0,50 ^c	06,64 \pm 0,06 ^b
	<i>Gboyébessé</i>	01,60 \pm 0,17 ^d	02,25 \pm 0,26 ^c	02,06 \pm 0,23 ^a	02,57 \pm 0,11 ^b	03,43 \pm 0,45 ^e	03,93 \pm 0,42 ^d
	<i>Gobi</i>	01,64 \pm 0,55 ^d	02,01 \pm 0,40 ^c	02,07 \pm 0,43 ^a	03,64 \pm 0,27 ^a	04,61 \pm 0,25 ^d	05,04 \pm 0,33 ^{bc}
	<i>Tongor</i>	01,45 \pm 0,21 ^d	02,11 \pm 0,38 ^c	01,09 \pm 0,13 ^d	01,55 \pm 0,06 ^d	04,64 \pm 0,35 ^d	05,07 \pm 0,37 ^{bc}
	ICRAD-I	01,12 \pm 0,16 ^d	01,34 \pm 0,09 ^d	01,92 \pm 0,10 ^b	02,50 \pm 0,09 ^b	02,86 \pm 0,07 ^f	03,19 \pm 0,30 ^d
	ICRAD-III	01,29 \pm 0,09 ^d	01,96 \pm 0,05 ^c	01,85 \pm 0,07 ^b	02,53 \pm 0,07 ^b	02,62 \pm 0,12 ^f	03,23 \pm 0,31 ^d
Water deficit	<i>Adibolo</i>	02,55 \pm 0,32 ^c	03,69 \pm 0,38 ^b	0,57 \pm 0,10 ^f	01,19 \pm 0,13 ^e	09,38 \pm 1,73 ^a	09,58 \pm 0,57 ^a
	<i>Gboyébessé</i>	02,64 \pm 0,41 ^c	03,34 \pm 0,18 ^b	0,57 \pm 0,05 ^f	01,21 \pm 0,15 ^e	06,60 \pm 0,32 ^b	07,29 \pm 0,58 ^{cd}
	<i>Gobi</i>	04,32 \pm 0,36 ^a	04,78 \pm 0,25 ^a	1,52 \pm 0,07 ^{bc}	02,65 \pm 0,36 ^b	06,29 \pm 0,63 ^b	06,90 \pm 0,29 ^b
	<i>Tongor</i>	03,43 \pm 0,34 ^b	04,81 \pm 0,17 ^a	0,80 \pm 0,11 ^e	01,15 \pm 0,05 ^e	06,33 \pm 0,63 ^{ab}	06,35 \pm 0,58 ^b
	ICRAD-I	03,34 \pm 0,13 ^b	03,56 \pm 0,09 ^b	01,48 \pm 0,14 ^{bc}	01,98 \pm 0,16 ^c	03,78 \pm 0,08 ^e	04,09 \pm 0,23 ^d
	ICRAD-III	03,70 \pm 0,09 ^b	04,21 \pm 0,08 ^a	01,33 \pm 0,08 ^{bc}	01,93 \pm 0,05 ^c	03,46 \pm 0,26 ^e	03,82 \pm 0,28 ^d

Digits with the same letter (s) in the same column are not significantly different at the 0.05 probability threshold (Newman-Keuls).

Table 4: Variation des measured parameters

The accumulation rates are relatively low in the cultivars Adibolo (60.38 % and 49.56 %) and Gboyébessé (66.70 % and 50.49 %) (Figure 3), far behind the cultivars Tongor (141.44 % and 133.60 %), Gobi (186.60 % and 144.28 %), ICRAD-I (204.06 % and 166.45 %) and ICRAD-III (188.93 % and 114.54 %).

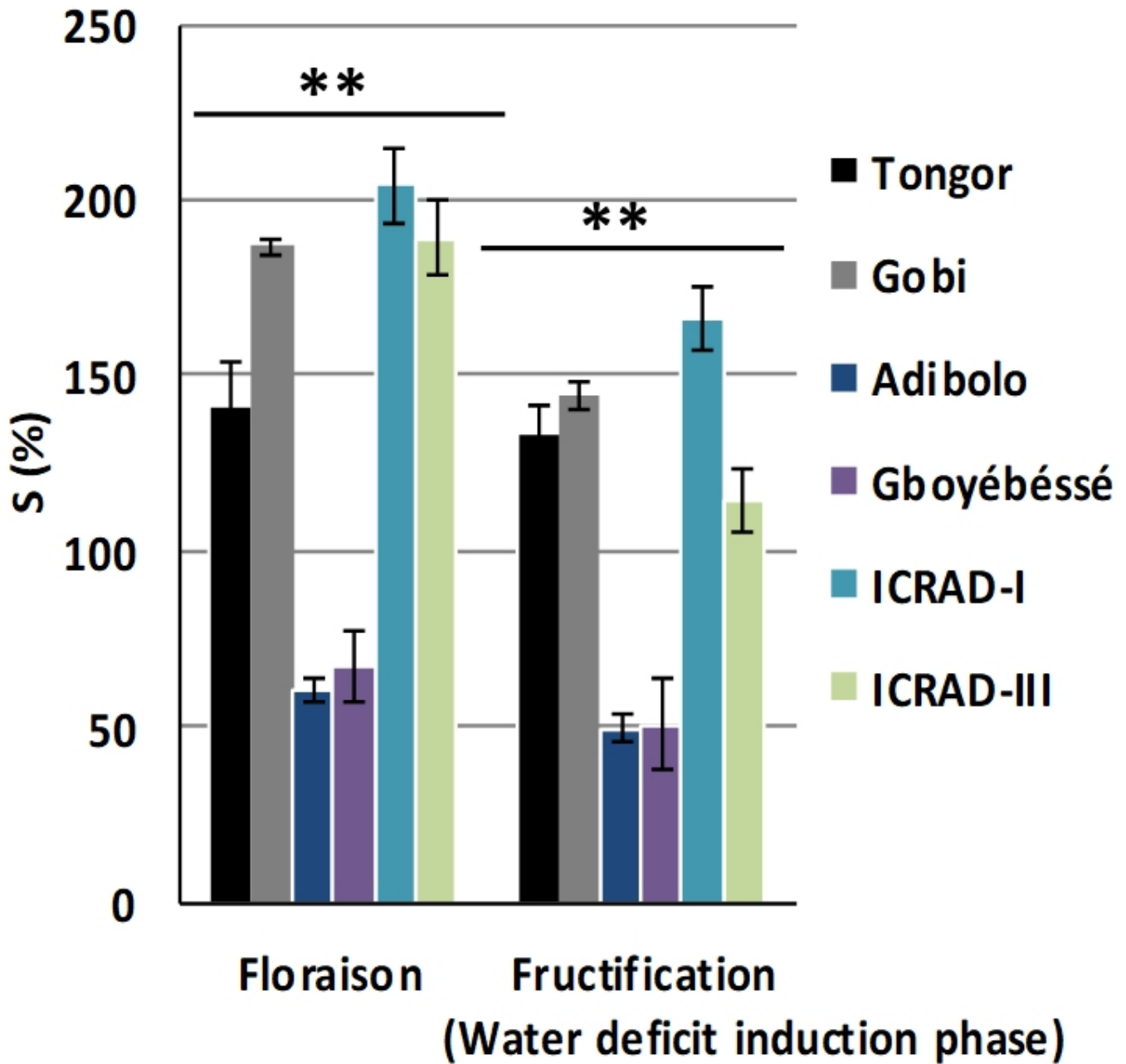


Figure 3: Proline accumulation rate in leaves under water deficit

Influence of water deficit on the total chlorophyll content in the leaves

The total chlorophyll contents were significantly reduced in water deficit regime (Table 4). All the cultivars were affected by water deficit, with however a more significant fall in the cultivars Adibolo and Gboyébéssé, respectively -71.24 % and -71.79 % at the flowering stage, -54.68 % and - 52.83 % at the fruiting stage, against only -26.87 %, -23.88 %, -22.55 % ,

-27.92 % at the flowering stage, and -25.89 %, -27.06 %, -20.71 % and -23.58 % at the fruiting stage respectively in the cultivars Tongor, Gobi, ICRAD-I and ICRAD-III (Figure 4).

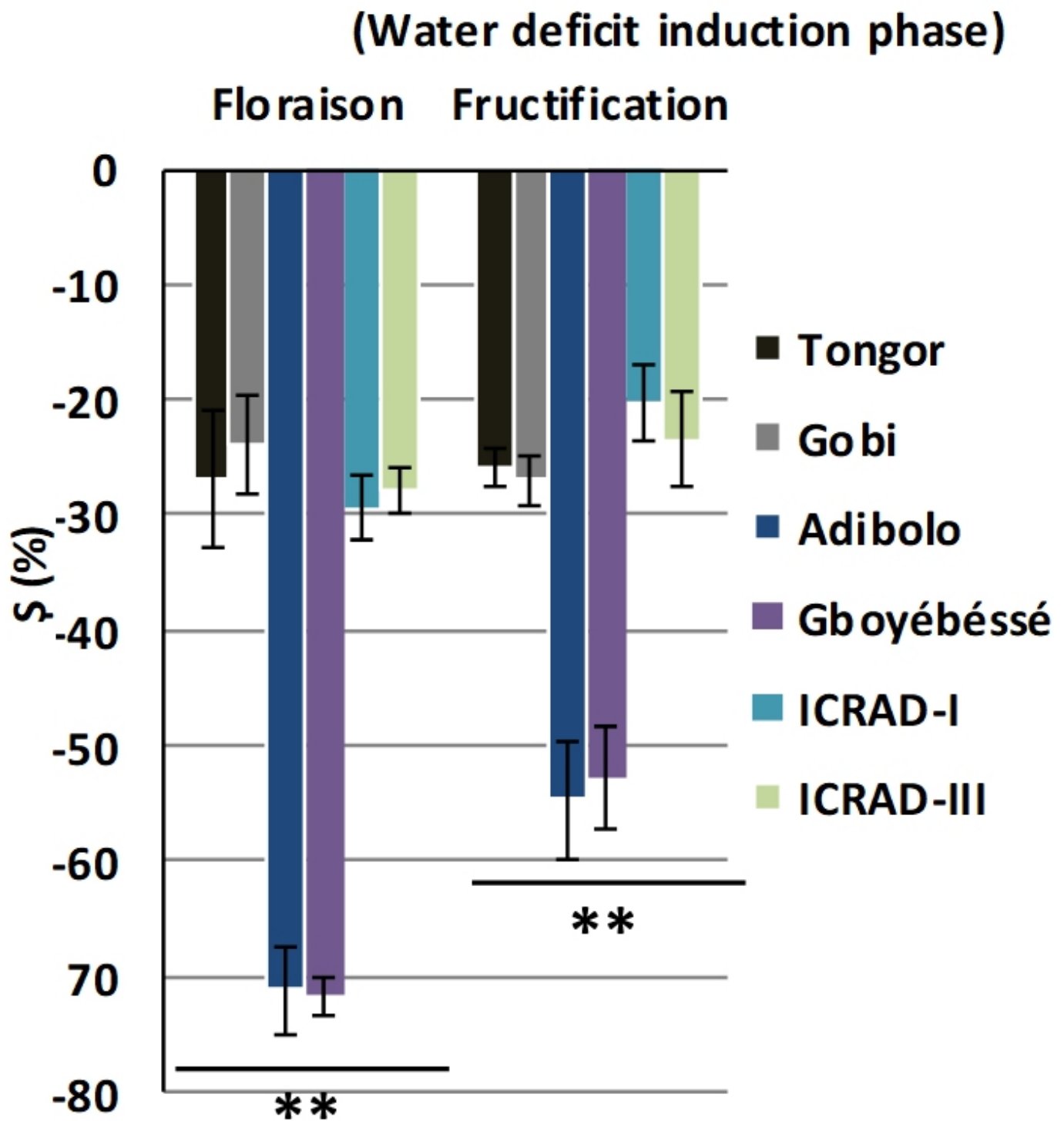


Figure 4: Chlorophyll pigment degradation rate in leaves under water deficit

Influence of water deficit on malondialdehyde (MDA) content in leaves

Water deficit induced an accumulation of MDA in the leaves in all the pepper cultivars studied (Table 4). The cultivars Gboyébessé and Adibolo seem by far the most affected by the oxidative stress related to water deficit, with accumulation rates of 94.59 % and 83.56 % respectively in the flowering phase and 87.26 % and 44.20 % in the fruiting phase (Figure V).

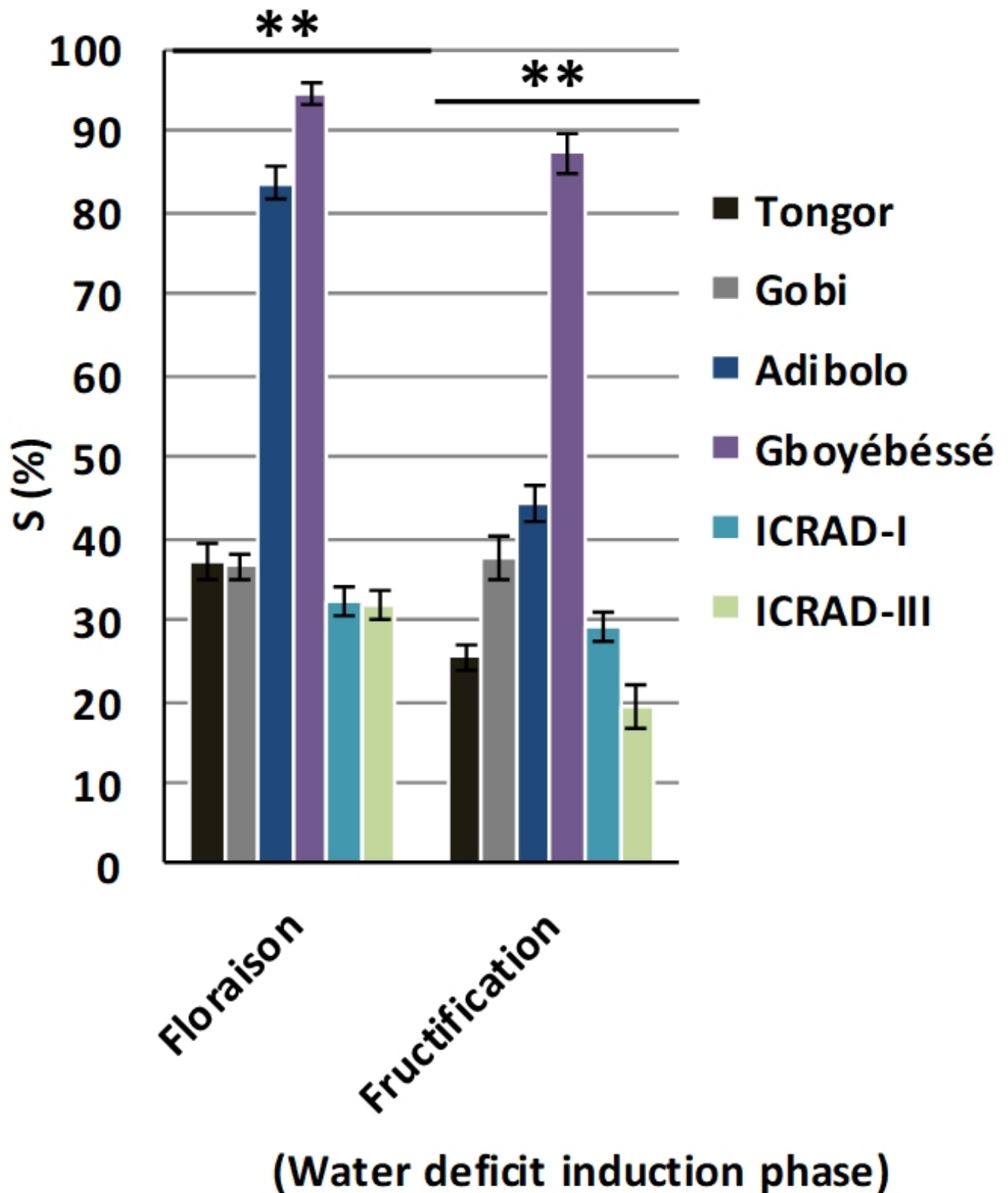


Figure 5: MDA accumulation rate in leaves under water deficit

Correlation analysis between the measured biochemical variables

In the case of lack of water, the analysis of the correlation matrix (Table 5) shows no significant correlation between the parameters measured, both at the flowering and fruiting stage of the

plants. Nevertheless, it is remarkable that the more the stressed plant accumulates proline, the less it concentrates malondialdehyde and experiences little degradation of the chlorophyll pigments.

Variables	Flowering stage			Fruiting stage		
	Proline	Chl	MDA	Proline	Chl	MDA
Proline	1			1		
Chl	0,921	1		0,963	1	
MDA	-0,972	-0,952	1	-0,991	-0,876	1

Digits in bold= Pearson correlation coefficient significant at 5 %; Chl.: total chlorophyll; MDA: malondialdehyde

Table 5: Correlation matrix (Pearson) between biochemical parameters under water deficit

DISCUSSION

The water deficit induced an accumulation of proline in the leaves. In fact, to maintain the balance of osmotic force, after the drop in water potential caused by water stress, plants accumulate a certain number of osmoticums such as proline (Wang et al., 2003) which, in association with other factors such as transpiration reduction (by stomata closing) and leaf area reduction (Karrou et al., 2001), keep the turgor and the cytosolic volume as high as possible (Bouzoubaa et al., 2001; Wang et al., 2003). The proline accumulation can be the result of three complementary processes: stimulation of its synthesis, inhibition of its oxidation and / or alteration of protein biosynthesis (Sandhya et al., 2010). The accumulated proline can intervene in the strengthening of the antioxidant system (Ma et al., 2006; Molinari et al., 2007) and could also be incorporated into the parietal proteins, allowing a reshaping of the wall for its strengthening (Ben Nja, 2014). Ouiza et al. (2010) were able to establish a positive correlation between the quantities of prolines accumulated and the level of tolerance of the plant to water deficit. Thus, according to the high proline accumulation rates in cultivars Gobi, Tongor, ICRAD-I and ICRAD-III, they can be considered as the most tolerant to drought out of the six cultivars studied.

The levels of chlorophyll pigments have decreased with the water deficit. These results confirm the observations of Kaya et al. (2010) on pepper under salt stress. This fall could be also the result of chlorophylls degradation by an oxidation of the intermediaries in chlorophyll biosynthesis pathway, or even, from an imbalance between the production of chlorophyll, amino acids and sometimes proteins (Eckhardt et al., 2004; Schelbert et al., 2009). Furthermore, since chlorophylls and proline have glutamate as a common precursor, increasing the proline content of chilli plants would disadvantage the accumulation of chlorophyll. Bousba et al. (2009) associate the fall in chlorophyll contents in conditions of lack of water with a reduction in the opening of stomata for water losses limitation by evapotranspiration and by increasing atmospheric CO₂ (necessary for photosynthesis) entrance.

Malondialdehyde (MDA) is considered to be a good indicator of plants tolerance to different abiotic constraints (Hernandez et al., 2000). Its determination provides information on cell membranes degradation level (Katsuhara et al., 2005). Peroxidation of membrane lipids is associated with an insufficient functioning of the detoxification system, which could lead to damage the main cellular components (Jiang and Huang, 2001). Furthermore, the increase in the content of leaf MDA could explain a decrease in photosynthetic activity. Indeed, lipid degradation would lead to a disruption of the thylakoid membranes, a loss of the integrity of the chloroplasts and consequently a decrease in photosynthetic activity (Benhassaine-Kesri et al., 2002).

In summary, two plant behaviors are observed under water constraints: (i) a varietal effect of water deficit, resulting in a lack of active osmotic adjustment (case of the cultivars Gboyébessé and Adibolo); (ii) a varietal effect of water deficit, resulting in an active accumulation of osmoticums such as proline (case of the cultivars Gobi, Tongor, ICRAD-I and ICRAD-III). These water-relation

variables are very important since they have an influence on the yield.

CONCLUSION

Drought is a period of low production due to the general sensitivity of all of the fundamental biological processes that determine plant productivity. At the end of this study, it is observed that water deficit has a significant impact on the physiology of the different pepper cultivars studied. Better drought tolerance is noted in cultivars Gobi, Tongor, ICRAD-I, ICRAD-III, while cultivars Adibolo and Gboyébessé are more sensitive. This variability of adaptive responses to drought can be exploited for a better control of the cultivation of chilli, especially in the irrigated system. However, it would be important to assess the best value of the different adaptation capacities on the yield components as well as the fruits quality.

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