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Improving water stress tolerance parameters in cotton (*Gossypium hirsutum* L.) using compost

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ABSTRACT

Objectives: Cotton is known for its sensitivity to water deficit. The aim of this study is to improve the water deficit resistance of this crop by applying compost to the soil.

Methodology and Results: The study was conducted in a greenhouse, in 96 vegetation pots of 10L containing either sandy soil or sandy soil with the compost. The device is a split-plot 4x4 with one plant per pot. Water deficit was induced at the flowering stage of the plant, for 30 days, and consisted of a decrease in irrigation from 70 % to 30 % of the useful water reserve (UWR). At the end of the water-deficient cycle, the contents of total proteins, proline, malondialdehyde, as well as the activity of catalase and ascorbate peroxidase were measured in leaf samples by spectrophotometry. Results showed an increase in the plant's tolerance to water deficit in the presence of compost in the growing soil, indicated by a significant accumulation ($p < 0.05$) of total proteins and proline, associated with increased activity of enzymes in the antioxidant system. Similarly, the use of compost has limited the oxidative damage caused by water stress, by a reduction in the accumulation of cellular malondialdehyde.

Conclusion and application of findings: Thus, producers could use composts made from agricultural residues to both increase productivity and protect plants from water stress.

Keywords: Cotton, water stress, tolerance, compost.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the cash crops generating huge incomes for producers and contributes to poverty reduction and social status improvement. Unfortunately, this crop faces many biotic stresses (pests, diseases) and abiotic stresses (drought, salinity). In tropical regions frequently constrained by drought episodes, the frequency and duration of which are expected to increase under the influence of climate change (IPCC, 2007), water stress remains a major factor influencing crop yield (Benamar *et al.*, 2009; Ibn Maaoui-Houimli, 2011). In Togo, agriculture is mainly rainy, and is

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characterized by an increasingly unfavourable environment pointed by irregular rains, pockets of drought at unexpected periods, temperature rises (Lemou, 2008; Badameli and Dubreuil, 2010; Adewi *et al*., 2010). The difficulty to water access, which is marked by a decrease in the amount of water per capita (FAOSTA, 2015), correlated with an ever-increasing population pressure, requires a more rational use of this commodity. For several years, soil fertility in cotton areas declined in Togo, mainly due to the gradual decrease in organic matter and mineral balance sheets deficit (Kintche *et al*., 2010). Overall, more than 50 % of cropland is degraded due to overexploitation and the loss of fallow in relation to population pressure (MERF, 2009). Chemical fertilizers are often used to restore soil fertility (around 16 % of farms in Togo have been treated with chemical fertilizers) but their increasing cost limits their use by the population. In addition, they are likely to have effects on the environment and on human and animal health. The sustainability of cropping systems depends on the rational management of soil fertility (Girma *et al*., 2007; Igué *et al*., 2008) which has become a major concern for cotton farms in Togo, due to the expansion of cotton cultivation and sub-optimal soil fertility management practices. Drought affects various physiological and biochemical processes in cotton, resulting in reduced growth, productivity and fibre quality (Kramer and Boyer, 1995). As water becomes limiting, the stomatic conductance decreases, resulting in a decrease in photosynthesis. Thus, carbohydrate metabolism is inhibited, leading to reduced plant growth and abscission of fruits (Loka *et al*., 2011). Effects of water stress on crops vary depending on the severity and duration of the stress, the stage of development of the plant, the considered genotype, and the interaction between these different factors (Flexas *et al*., 2006). However, facing water stress, the plant develops a range of mechanisms to mitigate stress effects, such as strengthening the antioxidant system, accumulation of heat shock and osmolyte proteins, and osmotic adjustment (Loka *et al*., 2011). In a dual context of water resource limitation and land degradation, it is important to propose technically effective and economically sustainable agricultural strategies, to mitigate the adverse effects of water stress and improve cotton tolerance to drought. Thus, the objective of this survey is to evaluate the effects of two types of composts made from agricultural residues from cotton areas, on some of the parameters characterizing cotton drought tolerance.

MATERIALS AND METHODS

Experimental site and plant material: The trial was conducted at "Centre de Recherché Agronomique de la Savane Humide" (CRA-SH) based in Kolokopé (Togo), from November 2017 to March 2018. The plant material consisted of cotton seed *Gossypium hirsutum* L. variety STAM129A. This variety was selected for testing because of its early maturity (130 days), high field productivity (2.5 $T.ha^{-1}$ seed cotton yield), good pest self-defence (Tozoou *et al*., 2015) and disease resistance (Akantetou, 2013). It is also the variety grown throughout Togo land.

Characteristics of the composts used: For the purpose of agricultural recovery of crop residues in cotton zones in Togo, different types of composts were developed as previously described (Gnofam *et al*., 2019). After windrowing, the heap was covered with a tarpaulin to keep the heat in and prevent insect reproduction. During the composting process, turning and watering are carried out every two weeks, in order to ensure a better mixing, an adequate aeration of the decomposing materials and a drying out which is detrimental to the good progress of biodegradations. The availability of the organic material deposit justifies the frequency

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of turning during the process. The contents of the heaps were watered to 50-70 % of the dry weight of the substrate (Finstein and Miller, 1985) estimated by the handle test, in order to maintain optimal conditions for decomposition. Process monitoring consisted of regular measurements of pile temperature (every 2 days) between 9:00 and 10:00 a.m., and of pile moisture (at each turnover). After a process that lasted 3 to 4 months depending on the types of residues composted, the composts were recovered and analyses on maturity and agronomic potential were performed.

Two composts, Cx (compost of cotton stalks + maize straw + goat and sheep manure + ashes) and Cy (compost of maize straw + sorghum straw + *Rottboellia cochinchinensis* straw) were retained because of their good level of maturity [NO_3/NH_4 (1.07 ± 0.05; 2.93 ± 0.07) and AH/AF (3.93 ± 0.32; 3.46 ± 0.59)], their richness in organic matter (28.97 ± 1.27 %; 32.02 ± 1.29 %) and in major fertilizing elements [N(1.47 ± 0.20 %; 1.33 ± 0.08 %); K(1.10 ± 0.01; 0.79 ± 0.007 %)], and their agronomic performance [plant size (50-57 cm); number of fruiting branches (9-10)] (Gnofam *et al*., 2019).

Growth conditions and induction of water stress: The culture substrate consisted of soil collected from the test site, sieved to 2 mm and sterilized by heating. The compost treatments received an amendment of 1/10, or 100 g of compost per 1 kg of soil (Tartoura, 2010; Bokobana *et al.*, 2019). After potting the growing substrates, a pre-irrigation was carried out every 2 days at the field capacity, until sowing on the 15th day. The survey was carried out under a greenhouse in 96 pots of 10 L (25 cm deep, 25 cm of upper diameter and 16 cm of lower diameter). Each pot was filled with 7 kg of substrate. The bottom of the pots was drilled with four (04) holes to allow the water to drain after watering. The device was a four-repetitive split-plot (Figure 1) with two interacting factors (fertilization and water regime). The experimental unit consists of four pots. The sowing was carried out at the rate of four grain per pot. At germination, irrigation was reduced to 70 % of the useful water reserve (UWR). The separation was achieved on the 21st day after sowing (DAS) to maintain one plant by pot. The induction of the water deficiency consisted of a decrease in irrigation, from 70 % of the UWR (control treatment) to 30 % of the UWR (stress treatment) from the 45th day DAS. The cycle of lack of water lasted 30 days, corresponding to the average duration of drought pockets in the full rainy season in Togo (Ledi *et al*, 2020). The UWR was calculated using the following formula (Baize, 2000; Bokobana *et al*., 2019):

$$UWR = (\Theta_{fc}\ 2.5 - \Theta_{wp}\ 4.2) \times T_{fine} \times E \times Da$$

UWR: useful water reserve; θfc 2.5: Moisture at field capacity in %, θwp 4.2: Moisture at permanent wilting point in %; Tfine: % in fine particles, E: soil depth in dm, Da: apparent soil density.

The irrigation of the plants is done by successive weighing of the pots, at a period of 3 days. During each weigh-in, the control is reduced to the same weight corresponding to 70 % of the UWR, while water-deficient treatments maintain the restriction of water content to 30 % of the UWR (Bokobana, 2017). Ambient air temperature and relative humidity were measured daily below the greenhouse at 8:00 a.m. / 2:00 p.m. / 5:00 p.m. throughout the test period. Average environmental conditions are: a 12-hour photoperiod, an average temperature of 24 °C / 35 °C / 20 °C at 8:00 a.m. / 2:00 p.m. / 5:00 p.m. and an average relative air humidity of 65 % / 41 % / 55 %, respectively. At the end of the water shortage cycle, leaf samples were collected for laboratory analysis.

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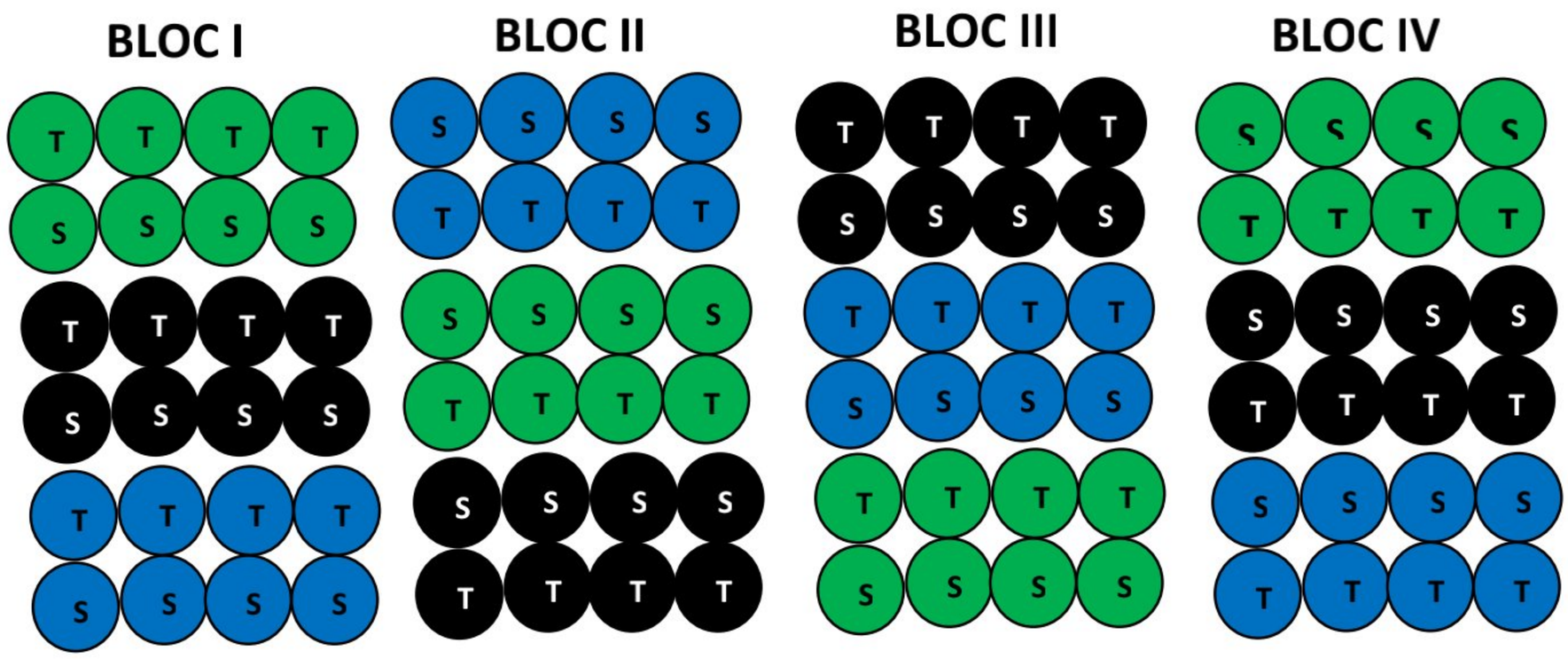

Figure 1. Test device (Split Plot 4 x 4)
UWR: useful reversion in water

Extraction and determination of total protein and antioxidant enzyme activities: Leaf tissues (0.5 g) were homogenized in 4ml of 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ascorbic acid and 1 % (w/v) polyvinylpyrrolidone (PVP) in an ice bath. The homogenate was centrifuged at 4 °C/ 14000 rpm for 10 minutes. The resulting supernatant was used for assays of total protein (Bradford, 1976) and activities of CAT (EC 1.11.1.6) and APX (EC 1.11.1.11) according to the method described by Parida *et al.* (2004).

Determination of leaf proline: Proline was determined according to Bogdanov *et al.*, (1999). 1 ml of formic acid (100 %) and 1 ml of ethylene glycol (3 %) were added to 0.5 mL foliar crude extract (25 mg/mL water) or standard proline solution (32 µg/mL).. After vigorous stirring for 15 minutes at room temperature, the mixture in the test tube is brought to a boil for 15 minutes. Then 2.5 mL 50 % 2-propanol is added to the mixture and incubated in a water bath at 70 °C for 10 minutes. After cooling the mixture at room temperature for 45 minutes, the absorbance is read at 510 nm with the spectrophotometer (type UviLine Connect Series 940) against a control sample made with distilled water. The leaf proline (TP) content, estimated as $\mu g.mg^{-1}$ protein, is then determined by the following formula:

$$\mathbf{TP = (Ae/As \times Ms/Mf) / QP}$$

Ae: Absorbance of leaf extract; As: Absorbance of standard proline solution; Ms: Proline mass of standard solution (µg); Mf: Fresh leaf mass (g); Qp: Protein quantity ($mg.g.^{-1}$ fresh material).

Extraction and determination of foliar malondialdehyde: Extraction and determination of MDA was done using the Heath and Paker (1968) method. As a result, 250 mg of fresh plant material was collected and crushed. The mill is homogenized in 5 mL of 5 % (w/v) trichloroacetic acid containing 1.25 % glycerol. The homogenate is centrifuged at 12 000 trs/min for 10 min and then filtered on Whatman N°1 paper. The supernatant was recovered in test tubes. 2 mL of 0.67 % thiobarbituric acid, prepared in

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distilled water, is added to 2 mL of supernatant. The mixture is homogenized in the vortex and incubated in a water bath at 100 °C for 30 min. After cooling in melting ice, the mixture was centrifuged again under the same conditions for one minute. The absorbance was measured at 532 nm and then at 600 nm. The optical density was then corrected by subtracting non-specific absorbance at 600 nm. The amount of MDA was calculated using a molar extinction coefficient of $155 mM^{-1}.cm^{-1}$.

Statistical analysis: Variance Analysis (ANOVA) was performed using XLSTAT software The Fisher test was used for the comparison of means when the analysis of variance revealed significant differences between treatments, at the 5 % threshold. A calculation of the rate of change (S) of the measured parameter is made according to the formula:

$$S = [(Vs / Vt - 1] \times 100$$

S: Rate of change of the measured parameter; Vs: Gross value of the stressed treatment; Vt: Gross value of the control treatment.

RESULTS

Change in total protein content: Water deficit induced a significant increase in total protein contents in the leaves (p = 0.0263) (Table 1). Total protein content was higher for plants grown on composts substrates than for those grown on the substrate without compost. The rates of increase were 438.73 % and 508.18 % for Cx and CY composts, compared to 283.44 % obtained without fertilization (Figure 2).

Table 1. Measures of biochemical parameters under water stress-induced

Water regime	Fertilization	Tot Prot. (mg.g^{-1}mf)	Prol (µg.mg^{-1} prot.)	MDA (mg.g^{-1} mf)	CAT (µmol H_2O_2.mg^{-1} prot.min^{-1})	APX (µmol ascorbate.mg^{-1} prot.min^{-1})
Normal irrigation	Without fertilizer	01.48±0.50^{d}	0.15±0.02^{e}	02.35±0.06^{b}	894.47±42.28^{a}	03.38±0.18^{ab}
	Compost Cx	02.41±0.49^{c}	0.32±0.03^{c}	02.02±0.20^{b}	602.45±58.57^{ab}	03.29±0.25^{ab}
	Compost Cy	02.44±0.77^{c}	0.25±0.02^{cd}	02.14±0.13^{b}	460.17±35.79^{bc}	02.89±0.24^{bc}
Water deficit	Without fertilizer	05.03±0.96^{b}	0.25±0.03^{cd}	03.76±0.13^{a}	358.32±83.45^{c}	04.16±0.17^{a}
	Compost Cx	12.61±0.71^{a}	0.94±0.12^{a}	02.52±0.21^{b}	141.13±31.64^{d}	04.92±0.31^{a}
	Compost Cy	14.11±01.53^{a}	0.62±0.12^{b}	03.01±0.20^{a}	193.18±11.01^{d}	04.55±0.13^{a}
	p	0.0263	0.0326	0.0268	0.0215	0.0322

Tot Prot.: Total proteins; Prol: Proline; MDA: Malondialdehyde; Chl.Tot: Total chlorophyll; CAT: Catalase; APX: Ascorbate peroxydase.

The numbers with the same letter(s) in the same column are not significantly different at the probability threshold of 0.05 (Fisher).

Cx: Compost of cotton stalks + maize straw + goat and sheep manure + ash, Cy: Compost of maize straw + sorghum straw + *Rottboellia cochinchinensis* straw.

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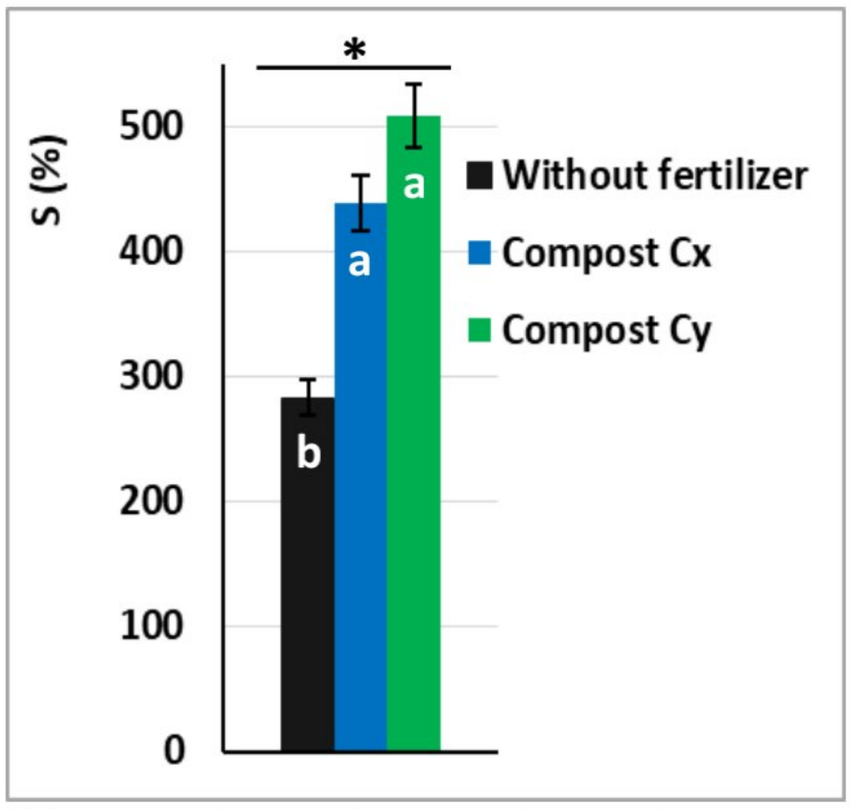

Figure 2. Rate of change in total protein content under water deficit.
* $P \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: non-significant.

Variation in foliar proline content: The proline content increased in cotton leaves (p = 0.0326) following the induction of water deficit (Table 1). This accumulation was higher in the plants grown on composts substrate (198.64 % with Cx compost and 151.15 % with Cy compost, compared to 70.67 % obtained without fertilization) (Figure 3).

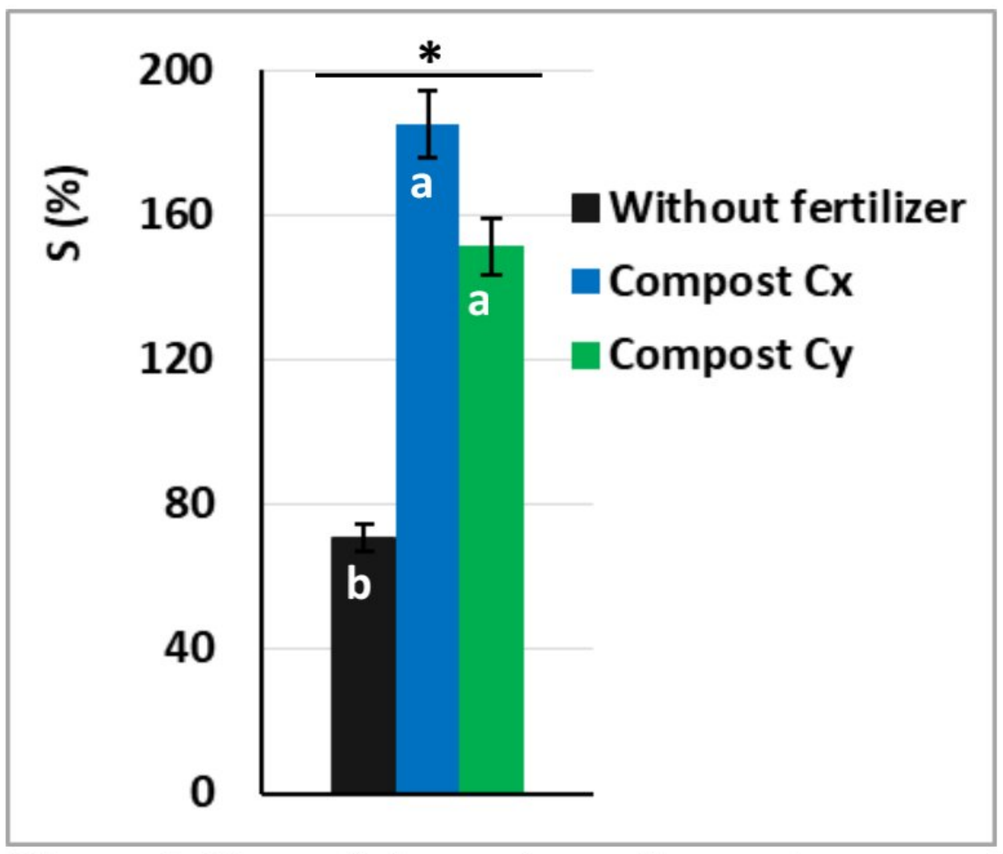

Figure 3. Rate of change in proline under water deficit.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: non-significant.

Variation in malondialdehyde content: Water deficit resulted in a significant accumulation of malondialdehyde in all treatments (p = 0.0268) (Table 1). However, this accumulation was lower for cotton plants grown on composts substrate (25.15 % for Cx compost and 40.75 % for Cy compost, than to 60.09 % in the absence of fertilization (Figure 4).

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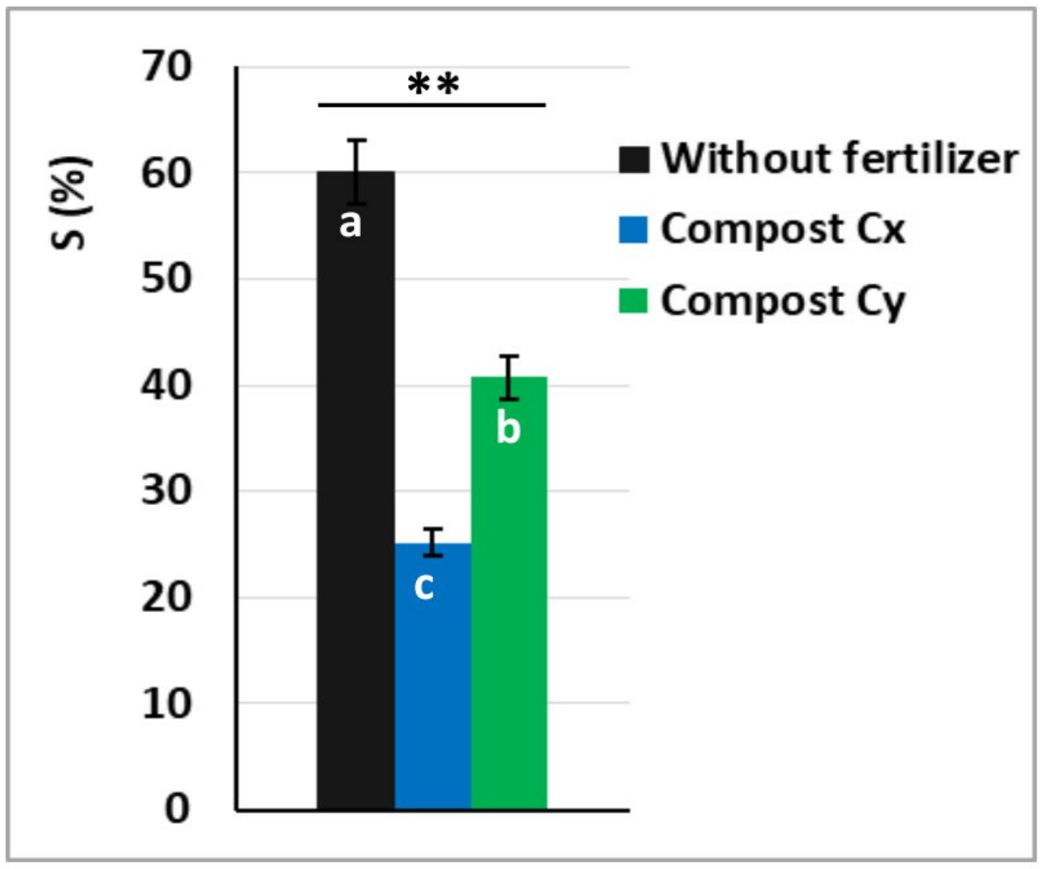

Figure 4. Rate of change in malondialdehyde content under water deficit.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: not significant.

Variation in activity of catalase and ascorbate peroxidase: Water deficit resulted in a significant decrease in catalase activity in all treatments (p = 0.0215), while ascorbate peroxidase activity increased significantly (p = 0.0322) (Table 1). For catalase, the rates of reduction of enzymatic activity were -60.09 % for the plants grown without compost, and -76.53 % and -60.42 % respectively with composts Cx and Cy (Figure 5). For ascorbate peroxidase, the rates of increase in enzymatic activity were high with composts, 50.51 % and 58.21 % respectively for compost Cx and compost Cy, compared to 23.19 % obtained without compost (Figure 6).

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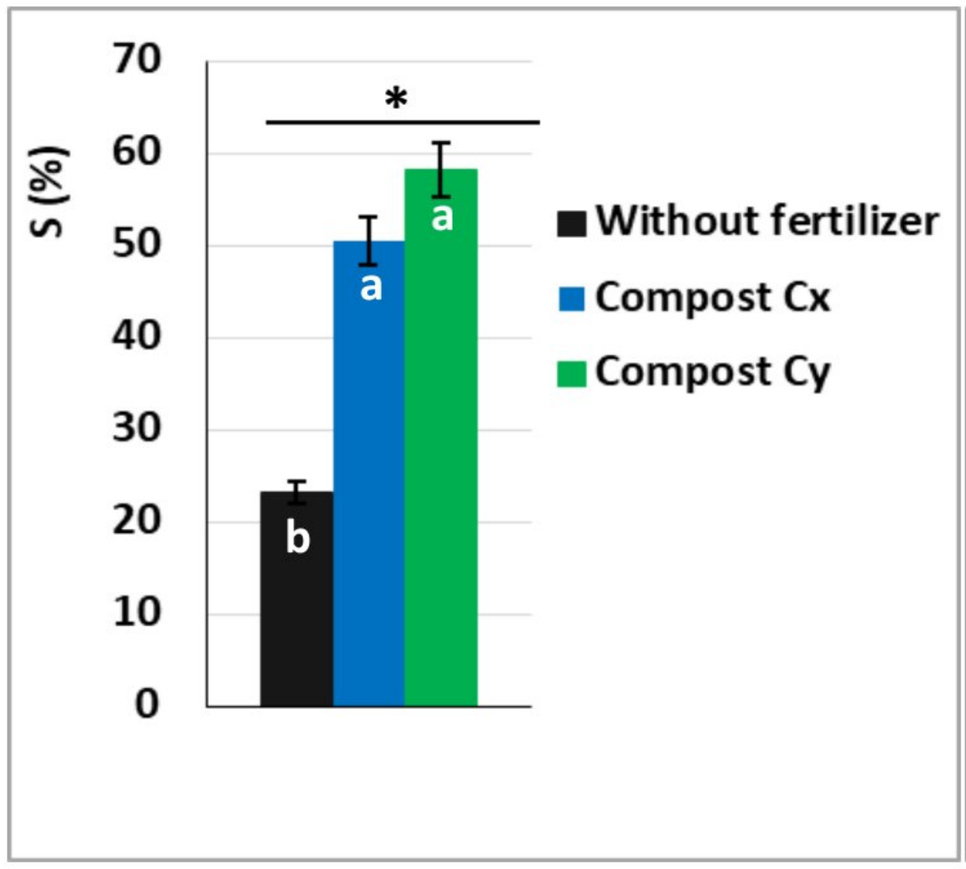

Figure 5. Rate of change in the activity of catalase under water deficit.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: not significant.

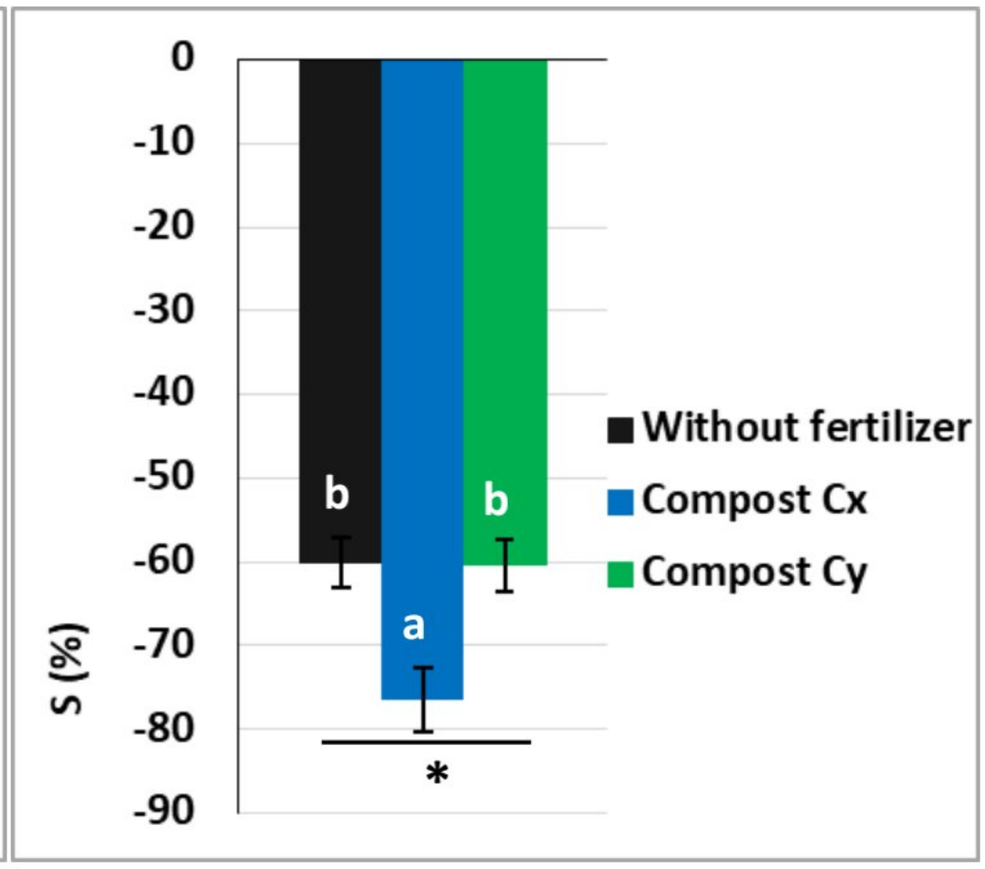

Figure 6. Rate of change in activity of ascorbate peroxidase under water deficit.
* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns: not significant.

DISCUSSION

Maintaining the integrity of the cell membrane and its stability under water deficit conditions is an important component of plant drought tolerance (Bajji *et al*., 2001). Malondialdehyde (MDA) is one of the products of the peroxidation of cell membrane lipids (Guichardant *et al*., 1994; Lacan and Baccou, 1998). Its accumulation is the symptom most attributed to oxidative damage. It is often used as a bio indicator of oxidative stress (Zhang and Kirkham, 1994). Therefore, MDA assay is an effective mean of assessing oxidative stress damage to the membrane (Katsuhara *et al*., 2005). Accumulation of MDA following application of water deficit is limited by soil fertilization by compost. Water deficit induced accumulation of proline in the leaves. In order to maintain the balance of osmotic force, after the loss of water potential due to water stress, plants accumulate a number of osmoticums, such as proline, which, in association with other factors like the reduction of perspiration by the closure of the stomata and the reduction of the foliar surface, allow to keep the turgescence and cytosolic volume as high as possible. Proline could also be incorporated into parietal proteins, allowing wall remodelling for reinforcement (Ouiza *et al*., 2010). Given this role of proline, accumulated amounts may be related to the water deficit tolerance level (Ouiza *et al*., 2010). Thus, the high accumulation rates in cotton plants grown on compost, prove that organic matter would increase the level of tolerance of the plant to water deficit (Tartoura, 2010; Some *et al*., 2010; Bokobana *et al*., 2019; Ledi *et al*., 2020). The accumulation of proline can be the result of three complementary processes: stimulation of synthesis, inhibition of its oxidation and/or alteration of protein biosynthesis. The accumulated proline would also play a role in strengthening the antioxidant system, indicating the strong positive correlations obtained between proline content and ascorbate peroxydase activity. CAT activity decreased while APX activity increased as a result of water deficit. These reflect the relationship between the two enzymes in excess cellular H_2O_2 catabolism. Indeed, water deficit may to some extent induce an excess of

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H_2O_2 in chloroplasts (Foyer *et al*., 2012) which may first inhibit CAT because it does not require a cell reducer (Kalefetoglu and Ekmekci, 2009), unlike APX (Mhamdi *et al*., 2010; Ouedraogo, 2013). When the CAT saturates with its substrate H_2O_2, it is subject to inactivation and the detoxification of H_2O_2 molecules can be relayed by APX whose activity increases. One of the main mechanisms for detoxifying ROS generated by stress conditions in plants is the Halliwell-Asada-Foyer or ascorbate-glutathione cycle (Foyer and Noctor, 2011). Thus, H_2O_2 is transformed by ascorbate peroxidase into water and oxygen. This ascorbate-glutathione cycle, which also neutralizes superoxide ions involves the intervention of several enzymes, including ascorbate peroxidase. In addition, some others have shown that redox reactions involving ascorbic acid are crucial for increasing plant tolerance to environmental constraints (Conklin and Barth, 2004; Halliwell, 2006; Dafré-Martinelli *et al*., 2011). Increased activity of antioxidant enzymes has been positively correlated with plant resistance to drought (Porcel and Ruiz-Lozano, 2004; Halliwell, 2006). According to the works of Verma *et al*. (2014) and Tartoura (2010), waste composts improve wheat's resistance to water deficit through an accumulation of enzyme such as APX, CAT and GPX, unlike plants grown on an unamended substrate. It is therefore clear that compost contributes to the improvement of cotton tolerance to the lack of water. The inactivation of CAT may induce de novo synthesis of the active enzyme by the cell, thereby contributing to an increase in the total protein content of stressed plants (Hearn *et al*., 1999; Polle *et al*., 2001; Asada, 2006). Total protein concentration increase, following the induction of water deficit has previously been reported in maize, wheat, rice (Abe *et al*., 2003; Zhu *et al*., 2004) It is due to the activation of a set of genes that allow the synthesis of specific proteins associated with stress such as Late Embryogenesis Abundant (LEA) proteins that protect the vital set of cellular proteins, and heat shock proteins (HSP) that support the protein and membrane structures of the plant cell (Iba, 2002). The effect of composts on biochemical stress indicators identified in this study may be associated with the presence in compost, macro and micronutrients (potassium, calcium, magnesium), humic substances, phytohormone-like substances, of biotic agents, nitric oxide, leading to the synthesis and accumulation of several metabolites (Akiyama *et al*., 2003; Raviv *et al*., 2004; Tejada *et al*., 2009; Jia *et al*., 2010; Bokobana *et al*., 2019). These various elements are contributed to plant resistance to frost, drought and disease, and to be important enzyme activators (FAO, 2005).

CONCLUSION

This study showed that soil-applied compost improved cotton's tolerance to water deficit by promoting the accumulation of proline and total proteins, and by enhancing the activity of antioxidant system enzymes (CAT, APX). Thus, the use of compost made from crop residues helps not only to restore degraded soils, but also to boost the resistance of crops to water stress. However, this improved resilience must be confirmed by assessing the impact of water stress on the productivity and quality of the harvested fibre of the plant.

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