Enhancement of proline content and antioxidant enzyme activities induced by drought stress in maize (Zea mays L.) by application of compost

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ABSTRACT

In sub-Saharan Africa, soil degradation and recurrent droughts are major obstacles to a sustainable agriculture. This study aimed at investigating the effect of compost addition to soil on proline content and activities of the antioxidant enzymes [catalase (CAT), ascorbate peroxidase (APX)] in maize plants, under drought stress conditions. The test was carried out in 20L plastic pots containing either sandy soil or sandy soil with the fertilizer, under natural conditions. The water deficit was induced at male blooming and milky grain stages. Plant irrigation was done by successive weighing of the pots during which the control is reduced to the same weight corresponding to 70% of the useful water reserve (UWR), while the stressed treatment maintains the water content at 30% of the UWR for 10 days. At the end of the stress period, the proline content, the CAT and APX activities in the leaves were determined through a spectrophotometry. The results show an important accumulation of proline and increase in enzymatic activity induced by water deficit in plants grown on compost (p = 0.00000 at p < 0.05). This study provides evidence for a beneficial effect of compost application in enhancing drought tolerance of maize.

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Keywords: Drought stress; compost; proline; antioxidant enzymes; Zea mays L.

INTRODUCTION

In recent decades, Togo has experienced serious deregulation of the rainfall, such as long-term droughts or floods at unexpected periods (Adewi et al., 2010; Lemou, 2008). Zones of drought appear during cultural cycles. From 1961 to 2010, great thermic increases have been registered in the southern and northern plains (respectively from 1, 0 to 1, 6 °C and 0, 9 to 1, 6 °C) of the country (Badameli and Dubreuil, 2010). The highest temperatures are registered almost each year and can reach 40 °C, more often in Maritime and Savannas regions (Badameli and Dubreuil, 2010). Average rainfall between 1986 and 2005 has decreased from 89 ± 22 mm in the whole country (DNM, 2007). From 1950 to 2000, about 27 days separate the early dates from the late dates of the start of the short rainy season in Guinean region (Adewi et al., 2010); this suggests that the potentially useful seasons has been almost inexistent in this part of the country from 1965. Thus, agricultural schedules are disturbed by an increasingly unpredictable climate, causing soil erosion and sometimes destroying plantations, with a negative impact on crop yields (Lemou, 2008;
Adewi et al., 2010). In some areas, crops such as maize dry before they even mature. In such a deadlock, the worst is predicted with an increasing risk of drought accompanied by a significant increase in the occurrence of extreme temperatures (IPCC, 2007).

In Togo, corn remains the first cultivated cereal crop, far in front of the sorghum, with 696 thousand hectares cultivated in 2014, for a production of about 833 044 tons (FAO, 2017). However, the effectiveness of its photosynthesis, its water efficiency and its drawing root system make corn a plant very subject to water deficiency, especially since it usually ends its cycle in a period which is critical in water (Barrière, 2001).

In water deficit situation, plants develop mechanisms of tolerance (osmoregulation, activity of the antioxidant system). The accumulation of proline is one of the most remarkable osmoregulation manifestations of water and osmotic stress (Demiral and Turkan., 2004). Moreover, in view of the accumulation of reactive oxygen species (ROS), which is the cause of an oxidative stress induced by a water stress, the capacity of the antioxidant system is crucial for maintaining the integrity of the photosynthetic system. One of the mechanisms of the antioxidant system is detoxification, which consists in preventing the accumulation of hydroxides by intervening at different stages of their formation. It is found in many enzymes such as superoxide dismutases (SOD), ascorbate peroxidases (APX), catalases (CAT), glutathione-S transferases (GST) and glutathione peroxidases (GPX) (Loka et al., 2011).

The purpose of this study was to assess the contribution of an organic amendment, a compost produced from a mixture of household waste, biomass of Cassia occidentalis L., cow manure, natural phosphate and ashes of cotton seeds, as an external factor in improving corn resistance to water deficit. More specifically, the effect of this compost on the accumulation of proline and the activity of CAT and that of APX is evaluated in corn in a situation of water stress at the stages of flowering male and milky grain of its development.

MATERIALS AND METHODS
Substrates analysis and composting materials
The sandy soil used for the purpose of the study was collected from the soil layer (0-25 cm depth) at the agronomic station of experiment of the Université de Lomé (6°22’N, 1°13’E; altitude = 50 m). It is an iron-type poor which pH was 5.6, EC (dSm⁻¹) was 1.87, total organic matter (TOM, %) was 1, total C, N, P, K in % are 0.8, 0.1, 0.11 and 0.026, respectively. The collected soil was air-dried, homogenized and sieved at 2 mm. The used compost was produced from a mixture of household waste, biomass of Cassia occidentalis L., cow manure, natural phosphate and ashes of cotton seeds at ratio of 5:7:1:2:5 (m/m) (Bokobana et al., 2017). The initial material of compost was mixed and was allowed to decompose in a tank. Moisture content was measured twice a week using a thermohygrometer and was maintained at 50–60% throughout the active composting period. The mixture was turned at 2, 4, 8, 16, 30, 60 and 90th day, to maintain porosity. The thermophilic stage (>55 °C) lasted for about 50 days. After 120 days of composting processes, the temperatures reached the ambient level. The compost was then left for curing about 4 weeks to be suitable as a culture medium. The compost pH and EC (dSm) were 6.25 and 4.44, respectively. TOM (%) was 47.64; total C, N, P and K (%) dried samples were 33.98, 1.73, 3.13 and 3.28, respectively. Humic and fulvic acids contents (%) were respectively 12.2 and 4.3. (Bokobana et al., 2017).

Plant growth and treatments
The test was undertaken in plastic pots of 20 L (31 cm deep; 31 cm in greater diameter; 21 cm in lower diameter) in which holes were made at the bottom (to let drain water after watering). 500 g of gravel were put down at the base to allow a good drainage of water. Every pot was filled with 18 kg of substratum. A pre-planting irrigation was applied every 2 days at the capacity on the fields, until the sowing on the 15th day. Maize (Zea mays L.) seeds, after keeping wet in the darkness for 72 hours, were planted, May 27, 2017 in pots (04 seeds/pot) containing sandy
soil amended with the compost (100 g/Kg soil) or with chemical fertilizer (1.5 g of NPK 15-15-15 and 0.75 g of urea), and grown along with control that received no fertilizer, under natural conditions. The separation which was achieved on the 14th day after sowing allowed to maintain one plant by pot. The environmental conditions were as follows: 12 h photoperiod, temperature of 26 °C / 29 °C / 27 °C 8 h / 14 h / 17 h and a relative humidity of 84% / 69% / 77%. During the growing period, the water content of the soil in all pots was maintained at 80% of the field capacity. The applied water deficit was about a lowering of the irrigation from 70% of the UWR (witness plant) to 30% of the UWR (stressed plant) at male bloom stage (right at the appearance of the panicle) and the milky grain stage (beginning of grains’ filling). The UWR (in mm) is given by the following formula:

\[ U.W. R = \sum_{k=1}^{n} \left( 0f_c 2.7 - \theta_{wp4.2} x T_{fine} x E x D_a \right) / h \]

h: Number of horizons, \( \theta_c \): Moisture at field capacity in %, \( \theta_{wp} \): Moisture at the wilting point in %, \( T_{fine} \): % fine soil, E: Soil depth in dm, Da: Soil apparent density.

The irrigation of plants has been made at a periodicity of 3 days, by a weighing of pots to maintain the desired soil water levels by adding appropriate volumes of water. The experimental treatments consisted of: controls [sandy soil without fertilizer] and five treatments [sandy soil amended with compost; sandy soil added with chemical fertilizer; sandy soil under drought stress; sandy soil amended with compost under drought stress; sandy soil added with chemical fertilizer under drought stress], arranged in a split-plot design with four replicates. The experimental unit consisted of 3 pots, giving a total of 108 pots (Figure 1). After 10 days of treatment, the youngest fully developed leaves were taken for biochemical assays.

**Assays of antioxidant enzyme activities**

Leaf tissues (0.5 g) were homogenized in 4 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM ascorbic acid and 1% (w/v) polyvinylpyrrolidone (PVP) in an ice bath. The homogenate was centrifuged at 4 °C/ 14 000 rpm for 10 minutes. The resulting supernatant was used for assays of the 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). The determination of protein content in the enzyme extract by the method of Bradford (1976) has permitted to calculate CAT and APX activities by the following formulas:

\[ \text{Act.} \text{CAT} = (\Delta \text{Abs} \times v) / (\varepsilon_{240} \times L \times \Delta t \times T \times m) \]

\[ \text{Act. APX} = (\Delta \text{Abs} \times v) / (\varepsilon_{290} \times L \times \Delta t \times T \times m) \]

\[ \text{Act. CAT}: \text{Catalase activity (} \mu\text{mol H}_2\text{O}_2, \text{mg}^{-1}\text{protein.min}^{-1}) ; \Delta \text{Abs}: \text{Average Difference in absorbance}; \varepsilon_{240}: \text{Molar linear extinction coefficient (39.4 mM}^{-1} \cdot \text{cm}^{-1}) ; \text{L: Diameter of the tank (cm)} ; v: \text{Volume of the reaction mixture (mL)} ; m: \text{Sample mass used for the enzymatic extract (g)} ; T: \text{Protein content (mg.g}^{-1}) ; \Delta t: \text{Culture time (min)}. \]

\[ \text{Act. APX}: \text{Ascorbate peroxidase activity (} \mu\text{mol ascorbate.mg}^{-1}\text{protein.min}^{-1}) ; \Delta \text{Abs}: \text{Average Difference in absorbance}; \varepsilon_{290}: \text{Molar linear extinction coefficient (34 mM}^{-1} \cdot \text{cm}^{-1}) ; \text{L: Diameter of the tank (cm)} ; v: \text{Volume of the reaction mixture (mL)} ; m: \text{Sample mass used for the enzymatic extract (g)} ; T: \text{Protein content (mg.g}^{-1}) ; \Delta t: \text{Culture time (min)}. \]

**Measure of leaf proline content**

The proline assay adapted to the leaf was performed according to Bogdanov method (1999). At 510 nm the absorbance of the leaf extract (25 mg.mL\(^{-1}\) of water) and the absorbance of standard proline solutions (32 µg.mL\(^{-1}\)) were taken to deduce the proline content (expressed in µg proline.mg\(^{-1}\) protein) in the extract. A 5 mL test tube contained 0.5 mL of standard proline or ground the leaf or water for the blank received 1 mL of formic acid 100% and 1 mL ethylene glycol 3%. After a vigorous mixing during 15 minutes at room temperature each tube was placed 15 minutes in a boiling bath. 2.5 mL of 2-propanol 50% were added in each tube then placed for 10 minutes in a water bath of 70 °C. When the tube took the room temperature 45 minutes later 1 mL was taken of its content of the absorbance reading at 510 nm with a
spectrophotometer. The proline content of the leaves, estimated in μg.mg⁻¹ of proteins, was determined by the following formula:

\[ P = \frac{(Ae/As \times Ms/Mf)}{Qp} \]

P: Proline content (mg.g⁻¹mf); Ae: Absorbance of the leaf extract; As: Absorbance of standard proline solution; Ms: Proline mass of the standard solution (μg); Mf: Leaf fresh mass (g); Proteins content (mg.g⁻¹ fresh matter).

**Dry aerial biomass measurement**

Plants were harvested on the 10 day of exposure to the drought when they were 90 days old. The dry aerial biomass (DAB) was measured after drying samples in an oven at 70 °C for 2 days.

**Statistical analysis**

The data were subjected to statistical analysis using STATISTICA software (version 6.0). Student Newman-Keuls test was applied to compare the treatment means. Any variable with a P value less than or equal to 0.05 is considered as significant. Two classification factors were used, the standard factor of fertilization and the stage of application of the water deficit. A calculation of the rate of change (S) of the measured parameter was performed.

\[ S = \frac{[(Vs – Vt) / Vt] \times 100} \]

S: Incidence of the water deficit; Vs: Value of stressed; Vt: Value of the witness.

**Figure 1:** Experimental device (Split Plot 3 x 4).

- **BLOC I:**
  - Without fertilization: 0
  - Compost: 1
  - Synthetic fertilizer: 2

- **BLOC II:**
  - Normal irrigation (70% of the RNW) 0
  - Water deficit at male bloom stage (30% of the RNW) 1

- **BLOC III:**
  - Water deficit at milky grain stage (30% of the RNW) 2
  - Normal irrigation (70% of the RNW) 0

- **BLOC IV:**
  - Water deficit at milky grain stage (30% of the RNW) 2
  - Normal irrigation (70% of the RNW) 0
RESULTS

The analysis of the variance (Tables 1 and 2) shows that the water regime, fertilization and interaction of both factors had a highly significant effect (p = 0.00000 to p <0.05) on the proline content and on the activity of CAT and APX at the leaf level. However, specificities in the variations of the variables measured are to be observed from one type of fertilization to another and following the phenological stage of the plant where the water deficit was applied.

Proline content of leaves

The proline content of leaves has significantly increased with the application of water deficit whatever the treatment (Table 3). Soil fertilization has significantly influenced the accumulation of proline at the foliar level. Indeed, this accumulation is higher in plants that are cultivated on compost (80.58 to 160.45%) than in those that are grown on chemical fertilizer (48.25 to 106.98%) (Figure 2A). Depending on the date of application of the water deficit, the accumulation rate of proline is significantly higher at the male flowering stage (106.98 to 160.45%) than at the milky grain stage (48.25 to 80.58%) (Figure 2B).

CAT and APX activities in the leaves

The water deficit led to an increase in catalase activity (Table 4) and ascorbate peroxidase activity (Table 5) in the leaves whatever the treatment. Depending on the type of fertilization, the rates of increase in catalase and ascorbate peroxidase activities remain significantly higher in plants grown on compost (22.71 to 75.34% for CAT activity; 57.35 to 63.08% for APX activity) than in plants grown on synthetic fertilizer (15.10 to 40.45% for CAT activity; 35.29 to 45.66% for APX activity) (Figures 3A and 4A). Depending on the date of application of water deficiency, only CAT activity remains significantly higher in the milky grain stage than in the male bloom stage (40.45 to 75.34% for milky grain stage; 15.10 to 22.71% for male bloom stage) (Figure 3B), when the differences in the APX activity remain non-significant (Figures 4A and 4B).

Analysis of the correlation

Under normal irrigation, the analysis of the correlation matrix (Table 6) show positive significant correlation between CAT and APX activities (r = 1.000; p = 0.014) at male bloom stage. In water deficit condition, and at male bloom stage, the analysis of the correlation matrix (Table 7) show positively significant correlation between CAT and APX activities (r =0.997; p = 0.048) and on the other hand between proline content and CAT activity (r = 1.000; p = 0.012) and APX activity (r = 0.998; p = 0.036). At milky grain stage, positive significant correlation was observed between CAT an APX activities (r = 0.998; p = 0.036), and on the other hand between CAT activities and proline content (r = 0.998; p = 0.042).

Dry aerial biomass

The analysis of the variances (Tables 8) shows that the water regime, fertilization and interaction of the both factors had a significant effect on dry aerial biomass. Water deficit significantly reduced dry aerial biomass (DAB) (Table 9). Compost crops were less affected by this decline, at only 2.63% and 2.25%, respectively, at the male bloom stage and the milky-grained stage (Figure 5A). On the other hand, the decrease in DAB is significantly greater for the water deficit applied to the male bloom stage, with decline rates respectively of 13.27%, 5.15% and 2.63%, compared with 8.22%, 2.31% and 2.25% for the water deficit applied to the milky-grained stage (Figure 5B).
Table 1: Analysis of the variance of proline content (Prol), catalase activity (Act. CAT) and ascorbate peroxidase activity (Act. APX) at the male bloom stage.

| Source             | dl | Prol.   | Act. CAT | Act. APX |
|--------------------|----|---------|----------|----------|
|                    |    | MS      | P        | MS       | P        | MS      | P    |
|                    |    | F       |          | F        |          | F       |      |
| WR                 | 1  | 0.48    | ***      | 150.95   | ns       | 0.13    | *    |
|                    |    | 39.6231 |          | 1.375681 |          | 12.16819|      |
| Fertilization      | 2  | 0.11    | *        | 1989.60  | **       | 0.59    | ***  |
|                    |    | 5.108608|          | 13.26523 |          | 35.50737|      |
| WR*Fertilization   | 5  | 9.95    | ***      | 5370.24  | ***      | 2.04    | ***  |
|                    |    | 379.9663|          | 37.83126 |          | 136.8648|      |

MS = mean square; dl: free level; WR = water regime; F= Fisher factor; P= significance; * = Significant at the 5 % threshold; ** = significant at 1% threshold; *** = significant at the 0.1 % threshold; ns = not significant.

Prol: proline content; Act. CAT: catalase activity; Act. APX: ascorbate peroxidase activity.

Table 2: Analysis of the variance of proline content (Prol), catalase activity (Act. CAT) and ascorbate peroxidase activity (Act. APX) at the milky grain stage.

| Source             | dl | Prol.   | Act. CAT | Act. APX |
|--------------------|----|---------|----------|----------|
|                    |    | MS      | P        | MS       | P        | MS      | P    |
|                    |    | F       |          | F        |          | F       |      |
| WR                 | 1  | 0.14    | *        | 276.95   | *        | 0.36    | **   |
|                    |    | 13.13461|          | 7.664747 |          | 29.17227|      |
| Fertilization      | 2  | 0.10    | ns       | 1850.31  | **       | 1.20    | ***  |
|                    |    | 2.705949|          | 16.25373 |          | 218.7752|      |
| WR* fertilization  | 2  | 3.42    | ***      | 31755.77 | ***      | 3.91    | ***  |
|                    |    | 68.35817|          | 245.7489 |          | 315.1543|      |

MS = mean square; dl: free level; WR = water regime; F= Fisher factor; P= significance; * = Significant at the 5% threshold; ** = significant at 1% threshold; *** = significant at the 0.1% threshold; ns = not significant.

Prol: proline content; Act. CAT: catalase activity; Act. APX: ascorbate peroxidase activity.

Table 3: Variation in proline content (mg.g⁻¹mf).

| Water regime | Fertilization | Male bloom stage | Milky grain stage |
|--------------|---------------|------------------|-------------------|
| Normal       | Without fertilization | 1.91 ± 0.11d     | 2.27 ± 0.09d      |
|              | Synthetic fertilizer | 2.18 ± 0.15cd    | 2.54 ± 0.24c      |
|              | Compost        | 2.21 ± 0.18cd    | 2.55 ± 0.21c      |
| Deficit      | Without fertilization | 2.39 ± 0.11e     | 2.54 ± 0.12e      |
|              | Synthetic fertilizer | 4.51 ± 0.26b     | 3.75 ± 0.23b      |
|              | Compost        | 5.73 ± 0.11a     | 4.60 ± 0.35a      |

Values with the same letter (s) in the same column are not significantly different from the probability threshold of 0.05 (Newman-Keuls).
**Figure 2:** Leaves proline accumulation rate under water deficit, (A) According to the type of fertilizer, (B) According to the date of application of the water deficit. S: rate of change; * P≤0.05 ** p≤0.01; *** p≤ 0.001; ns: not significant.

**Table 4:** Variation in CAT activity (µmol H$_2$O$_2$. mg$^{-1}$.prot.min$^{-1}$).

| Water regime | Fertilization     | Male bloom stage | Milky grain stage |
|--------------|-------------------|------------------|-------------------|
| Normal       | Without fertilization | 1.41 ± 0.12$^c$ | 1.55 ± 0.06$^c$ |
|              | Synthetic fertilizer | 1.97 ± 0.18$^c$ | 2.12 ± 0.09$^d$ |
|              | Compost           | 2.14 ± 0.05$^c$ | 2.64 ± 0.06$^c$ |
| Deficit      | Without fertilization | 1.66 ± 0.08$^d$ | 1.97 ± 0.14$^d$ |
|              | Synthetic fertilizer | 2.66 ± 0.16$^b$ | 3.09 ± 0.16$^b$ |
|              | Compost           | 3.37 ± 0.10$^a$ | 4.31 ± 0.11$^a$ |

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

**Table 5:** Variation in APX activity (µmol ascorbate. mg$^{-1}$.prot.min$^{-1}$).

| Water regime | Fertilization     | Male bloom stage | Milky grain stage |
|--------------|-------------------|------------------|-------------------|
| Normal       | Without fertilization | 20.09 ± 1.24$^d$ | 21.05 ± 0.81$^d$ |
|              | Synthetic fertilizer | 23.31 ± 1.38$^c$ | 24.17 ± 1.28$^c$ |
|              | Compost           | 24.38 ± 1.03$^c$ | 25.18 ± 1.06$^c$ |
| Deficit      | Without fertilization | 20.96 ± 0.81$^d$ | 22.23 ± 0.26$^{cd}$ |
|              | Synthetic fertilizer | 26.79 ± 0.89$^b$ | 33.83 ± 1.60$^b$ |
|              | Compost           | 29.88 ± 1.60$^a$ | 44.12 ± 1.30$^a$ |

Values with the same letter (s) in the same column are not significantly different from the probability threshold of 0.05 (Newman-Keuls).
Table 6: Correlation matrix (Pearson) between biochemical variables under normal irrigation.

| Variables     | Male bloom stage | Milky grain stage |
|---------------|------------------|-------------------|
|               | Prol. Act.CAT    | Act.APX           | Prol. Act.CAT Act.APX |
| Prol.         | 1                | 1                 |
| Act.CAT       | 0.986            | 1                 |
| Act.APX       | 0.990            | 1.000             |

Numbers in bold = significant Pearson correlation coefficient at 5%

Prol.: proline content; Act. CAT: catalase activity; Act. APX: ascorbate peroxidase activity.

Table 7: Correlation matrix (Pearson) between biochemical variables under water deficit.

| Variables     | Male bloom stage | Milky grain stage |
|---------------|------------------|-------------------|
|               | Prol. Act.CAT    | Act.APX           | Prol. Act.CAT Act.APX |
| Prol.         | 1                | 1                 |
| Act.CAT       | 1.000            | 1                 |
| Act.APX       | 0.998            | 0.997             |

Numbers in bold = significant Pearson correlation coefficient at 5%

Prol.: proline content; Act. CAT: catalase activity; Act. APX: ascorbate peroxidase activity.

Table 8: Analysis of the variance of dry aerial biomass (DAB) at the different stages.

| Source             | Male bloom stage | Milky-grained stage |
|--------------------|------------------|---------------------|
|                    | dl               | MS                  | P       | F      | MS       | P    | F      |
| WR                 | 1                | 380.81              | **      | 53.32  | 146.42   | ***  | 24.39  |
| Fertilization      | 2                | 9974.70             | ***     | 606.57 | 9974.70  | ***  | 606.57 |
| WR*Fertilization   | 5                | 7465.32             | ***     | 578.22 | 7807.57  | ***  | 628.83 |

MS = mean square; dl: free level; WR = water regime; F= Fisher factor; P= significance; *= significant at the 5% threshold; **= significant at 1% threshold; ***= significant at the 0.1% threshold; ns = not significant.

Table 9: Variation in dry aerial biomass (g).

| Water regime   | Fertilization       | DAB (g)     |
|----------------|---------------------|-------------|
|                | Without fertilization| 135.95 ± 3.48d |
|                | Synthetic fertilizer| 220.12 ± 3.74b |
|                | Compost             | 233.79 ± 1.42a |
|                | Without fertilization| 120.02 ± 1.47f |
|                | Synthetic fertilizer| 209.4 ± 6.19f  |
|                | Compost             | 227.82 ± 2.97a |
| Deficit at Male bloom stage | Synthetic fertilizer| 219.18 ± 1.42b |
|                | Compost             | 232.95 ± 3.65a |
| Deficit at Milky-grained stage | Without fertilization| 129.90 ± 3.14d |
|                | Synthetic fertilizer| 219.18 ± 1.42b |
|                | Compost             | 232.95 ± 3.65a |

Values with the same letter (s) in the same column are not significantly different from the probability threshold of 0.05 (Newman-Keuls). DAB= dry aerial biomass.
Figure 3: Catalase activity increase rate under water deficit, (A) according to the type of fertilizer, (B) according to the date of application of the water deficit. S: rate of change; * P≤0.05; ** P≤0.01; *** P≤ 0.001; ns: not significant.

Figure 4: Ascorbate peroxidase activity increase rate under water deficit, (A) according to the type of fertilizer, (B) according to the date of application of the water deficit. S: rate of change; * P≤0.05; ** P≤0.01; *** P≤ 0.001; ns: not significant.
Figure 5: Dry aerial biomass decrease rate under water deficit, (A) according to the type of fertilizer, (B) according to the date of application of the water deficit. S: rate of change; * P≤0.05; ** p≤0.01; *** p≤ 0.001; ns: not significant.

DISCUSSION
Proline accumulation under drought conditions may 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 could play a role of osmoticum (Demiral and Turkan, 2004), and would intervene in the strengthening of the antioxidant system and the fight against stress damage (Molinari et al., 2007). This indicates the high positive correlations between the proline content and the activity of catalase and ascorbate peroxidase. Proline could also be incorporated into parietal proteins, allowing remodeling of the wall for strengthening (Ben Nja, 2014). It could also play a role in the regulation of cytoplasmic pH or constitute a nitrogen reserve used by the plant after the period of water deficit (Kavi Kishor et al., 2005). Given this role devoted to proline, the accumulated quantities could be linked to the level of tolerance to the water deficit (Ouiza et al., 2010). Thus, the high accumulation rates in plants grown on compost are proof that organic matter would raise the tolerance level of the plant to water deficit (Tartoura, 2010; Some et al., 2010).

Water deficiency induced an increase in the activity of catalase and ascorbate peroxidase. Similar results have been reported by many researchers in other abiotic stress studies (Caverzan et al., 2012; Sofo et al., 2015). These results indicate that both enzymes are involved in the cellular H$_2$O$_2$ catabolism that is harmful to cellular integrity (Meksem, 2007). The rates of increase in catalase and ascorbate peroxidase activity remained significantly higher in plants grown on compost than in plants grown on chemical fertilizer. Shigoeka et al. (2002) demonstrate a close relationship between the increase in ascorbate peroxidase activity and the intensity of water deficiency in the cell. The increased enzyme activity of the antioxidant system was positively correlated with plant resistance to drought (Halliwell, 2006). Moreover, it’s found that drought-resistant crop strains had higher levels of antioxidant enzymes than drought-sensitive strains (Laxa et al., 2019). According to Tartoura (2010) and Verma et al. (2014), waste compost improves wheat resistance to water deficiency through an accumulation of enzymes such as APX, CAT and GPX, unlike plants grown on a substrate without amendment. Thus, organic amendments, through their composition in micronutrients and organic matter, would
strengthen the defense system developed by plants in a situation of water stress.

The effect of composts on the biochemical stress indicators found in this study would be associated to the presence in the compost of macro and micronutrients (magnesium, calcium, potassium), organic matter, phytohormone-like substances, biotic agents, carbon dioxide, nitric oxide and many others, leading to the synthesis and accumulation of several metabolites (Jia et al., 2010). In addition to nutrients and organic matter, compost contains a significant amount of humic substances (Francou, 2003). Humic substances are natural organic polyelectrolytes (Chen et al., 2004a). Among the various functional actions of humic substances, their ability to improve plant growth has been well established in various species (Chen et al., 2004b). However, the mechanism behind the action of humic substances is poorly understood. While some authors suggest that humic substances promote plant growth by improving the bioavailability of certain nutrients in soil, mainly iron and zinc (Chen et al., 2004a; Chen et al., 2004b), others suggest that humic substances can also directly affect plant metabolism (Some et al., 2010).

The variability of the biochemical parameters measured from one phenological stage to another could be associated on the one hand with the physiological age of the plant (senescence) but on the other hand with the growth conditions (Valentinuz and Tollenaar, 2004). During the filling phase of the grains, the lack of water causes an accelerated senescence of the leaves.

The study showed also that corn further reduces its aboveground biomass under dry conditions. Similar results have already been obtained by Meskelu et al. (2014). This reduction is explained by Imorou et al. (2018) by reducing the leaf area (photosynthesis site) and stem elongation, by decreasing the number of grains due to poor fertilization (at bloom stage) or by the reduced accumulation of starch in the endosperm (at the milky-grained stage). However, this decrease in aerial biomass remains low for compost crops in favor of grain production and filling (Imorou et al., 2018).

Conclusion
The analysis of biochemical indicators of water stress in maize accounts for the effect of fertilization on the metabolism linked to the plant's resistance to drought. Thus, the synthetic voices of osmolytes (proline) in the fight against the effects of water stress, as well as the activity of the enzymes of the antioxidant system (CAT and APX) would be significantly stimulated by chemical factors present in organic matter. This would considerably reduce the damage caused by water stress to the development of the plant. This study shows that, besides improving the physical and chemical properties of the soil, compost offers the plant a better metabolic predisposition to withstand the effects of drought. However, the evaluation of the performance components can validate the metabolic flexibility obtained in this chapter.

COMPETING INTERESTS
The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS
All authors contributed to the realization of this work. They also read and approved this manuscript.

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