Glyphosate Plus Carboxylic Compounds Boost Activity of Free Radical-Scavenging Enzymes in Sugarcane

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Abstract: Drought, heat, and salinity, as well as pests, are stressing agents, which have impressively declined the productivity and quality of sugarcane crop in harsh environments. Our study aimed to examine the effect of various chemical ripeners as alternatives to enhancing the reactiveness of the enzymatic antioxidant system of sugarcane crop. The field experiment consisted of spraying the ingredients, ethephon, ethyl-trinexapac, glyphosate, carboxylic compounds (MTD) and methyl-sulfumeturon on the Brazilian commercial varieties, SP80-1842 and SP80-3280, before flowering stage. The enzymatic assay comprised the monitoring of the rate of degradation of free radical by ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) in the extract from leaves of 11-month-old plants. Spraying glyphosate at 0.15 L ha$^{-1}$ with MTD at 1.00 L ha$^{-1}$ provided the highest activity of CAT, 0.65 µmol H$_2$O$_2$ min$^{-1}$ mg$^{-1}$ protein, in variety SP80-1842 Spraying glyphosate at 0.15 L ha$^{-1}$ with ethephon at 0.33 L ha$^{-1}$ caused the highest activity of APX, 1.70 nmol ascorbate min$^{-1}$ mg$^{-1}$ protein, in variety SP80-3280. The conclusion is, therefore, that mixtures of glyphosate with the insecticide/acaricide, MTD, and with the synthetic ethylene-releasing product, ethephon could help sugarcane crop grow adequately under uncontrollable or unpredictable agroecosystems like marginal lands.

Keywords: induced oxidative stress; plant growth regulators; Saccharum sp.

1. Introduction

Experts in systematics and taxonomy classify sugarcane (Saccharum sp.) into the family Poaceae. Long, narrow leaf structures and relatively large, branched root system are relevant morphophysiological features of sugarcane to dynamically capture energy resources from the atmosphere and soil, then, stock them, and convert them into useful biomass through the path of photosynthesis. Sugarcane crop is replete of strengths. Economically, it provides the staggeringly rising world population with food, biofuels for transportation and feeding of power plants, as well as fine chemicals. Further benefits of sugarcane for agriculture include the production of silage and...
forage for ruminants and non-ruminants, and the real possibility of recycling of filter cake and stillage into harmless, inexpensive bio-fertilizers [1,2]. Environmentally, it is one of the most physiologically effective, most reliable and most affordable biosystems in mitigating the emissions of greenhouse gases into the atmosphere. Socially, it has great potential in creating employment opportunities and, consequently, modernize rural communities across low-income and high-income countries [3].

Brazil, India, the People’s Republic of China and Thailand account for the top producers of sugarcane in the world. In Brazil, fields of sugarcane extend for roughly 10 million hectares. Domestically, the state of São Paulo impressively holds over 50% of production of anhydrous and hydrated alcohol [4]. Nevertheless, industrial decentralization and opening of agricultural frontiers across national territory are factors driving the milling plants, biorefineries, and distilleries to migrate from Southeast towards the Midwest and Northeast. Drought, heat, salinity, and pests are the major abiotic and biotic stressing agents declining the productivity and quality of extensive plantations of sugarcane in the Midwest and Northeast, tropical regions where huge requirements of expensive inputs, such as irrigation, fertilizers, and pesticides, make the total cost of production and market price of products to some extent high, thus, threatening the sustainability of sugar-energy sector [5–7]. The development and implementation of strategies to enable producers adequately grow sugarcane under marginal lands are, therefore, necessary for both technical and economic reasons [8].

In crops under stressing conditions, endogenous levels of reactive oxygen species (ROS) substantially increase, thus, overwhelming the redox potential of cells. Anion superoxide (O$_2^-$), singlet oxygen (O$_2^*$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are typical free radicals deterring the perfect functioning of physiological and biochemical pathways of synthesis of key elements for plant growth and development, such as nucleic acids, ATP, proteins and hormones [9,10]. Events of oxidative stress lead the plant to undergo lipid peroxidation, degradation of photosynthetic and photoprotective pigments, like chlorophylls and carotenoids, disruption of the polar membrane, dehydration, and other harmful reactions. To fight ROS, the plant triggers the defence system, which includes several enzymatic and non-enzymatic substances. Ascorbic acid, flavonoids, aldehydes, alkaloids, vitamin E and alpha-tocopherol are non-enzymatic antioxidant substances, while the ascorbate peroxidase, catalase, peroxidase and superoxide dismutase are few of the most effective ROS-scavenging enzymes. ROS is not necessarily completely negative. While it appears that excessive concentration of ROS damages the plant, low levels are part of signaling mechanisms that acclimate to biotic and abiotic stresses [11–13]. Experts seriously rely on the enhancement of the plant stress defense system as the real key to providing agriculture with productivity and quality of food, energy, fiber and raw materials [14–19].

Scientific studies on the effects of stressful factors on the physiological, histological and developmental behavior of crop plants report ascorbic acid, polyethylene glycol and herbicides are organic and inorganic substances, highly effective in making plant stress defense system healthier and extremely reactive to free radicals [20–22]. Chemical ripeners could, therefore, be potential attempts to protect plantations of sugarcane under marginal lands from eventual adversities of drought, heat, salinity, etc. According to our knowledge, chemical ripeners, sometimes known as phytoregulators or plant growth regulators, refer to synthetic substances controlling the growth and development of the plant. Technically, they can be stimulants or delayers of cellular division and elongation. Irrespective of the category, phytoregulators act similarly to natural endogenous hormones, such as auxins, gibberellins, abscisic acid, ethylene, salicylic acid, indole-acetic acid, jasmonates, etc. Ethephon, ethyl-trinexapac, glyphosate, and methyl-sulfumeturon are ripening chemicals regular in Brazil’s sugar-energy sector. Such ingredients play a key role as management strategies, not only to improve productivity and quality of feedstock, but also to make it physiologically suitable for mechanical harvesting as earlier as possible, with higher sucrose to fiber ratio, thus, saving costs of production later in the season. Most of the country’s producers from the regions, Southeast, Midwest, and Northeast, traditionally spray these ingredients on the fields before flowering stage—the goal is to concentrate soluble carbohydrates homogeneously in the whole stalk by inhibiting auxins and
gibberellins signaling for the promotion of growth and development of vegetative structures, such as leaves and roots. The use of chemical ripeners is highly efficient in plantations of sugarcane under uncontrollable or unpredictable conditions for natural ripening. Despite advantages, phytoregulators at an excessive level generally fail to ripen sugarcane adequately, while causing oxidative stress as a consequence [23,24].

The objective of this study was to examine various chemical ripeners as alternatives to enhancing the reactivity of the enzymatic antioxidant system in sugarcane crop.

2. Materials and Methods

2.1. Site

We performed the field experiment at the Santo Antonio Farm, state of São Paulo, Southeast Brazil, during the 2015–2016 growing season. The soil of the area is Oxisol, with sandy-loamy texture. According to the Köppen-Geiger system, the regional climate is Aw, with predominantly rainy summer and dry winter, with annual averages of temperature, the relative humidity of the air and rainfall equivalent to 21.6 °C, 70%, and 1344 mm, respectively.

2.2. Chemical Ripening Agents and Varieties of Sugarcane

To investigate the technical viability of the ripening chemicals, ethephon, ethyl-trinexapac, glyphosate, MTD and methyl-sulfometuron, we selected the commercial varieties, SP80-1842 and SP80-3280. Producers from Southeast Brazil usually grow these varieties to provide the milling plants, biorefineries and distillers with suitable raw material for the production of white sugar, jaggery, syrup and bioethanol, and co-generation of heat and bioelectricity from burning lignocellulose in high pressure and temperature furnace-boiler systems. Table 1 summarizes the characteristics of the ingredients, registered from the Ministry of Agriculture, Livestock and Food Supply (MAPA) [25].

| Active Ingredient         | Label            | Class                      |
|---------------------------|------------------|----------------------------|
| Ethephon                  | Ethrel           | Plant growth regulator     |
| Ethyl-trinexapac          | Moddus           | Plant growth regulator     |
| Glyphosate                | Roundup Transorb| Systemic herbicide         |
| Carboxylic compounds      | MTD              | Insecticide/Acaricide      |
| Methyl-sulfometuron       | Curavial         | Plant growth regulator     |

2.3. Experimental Planning

The experiment was established in a randomized complete block design (RCB) with a 9 × 2 factorial scheme (9 treatments by 2 varieties) and 5 replicates per treatment as shown in Table 2.
Table 2. Experimental tests planned for the investigation of technical viability of the chemical ripening agents as low-cost strategies to enhance enzymatic antioxidant system of sugarcane crop.

| Test | Composition of Spray Solution * | Variety |
|------|--------------------------------|---------|
|      | Active Ingredient I | Active Ingredient II |
| 01   | Ethephon at 0.66 L ha⁻¹ | SP80-1842 |
| 02   | Ethyl-trinexapac at 0.80 L ha⁻¹ ** | - |
| 03   | Glyphosate at 0.35 L ha⁻¹ | - |
| 04   | MTD at 1.00 L ha⁻¹ | - |
| 05   | Methyl-sulfumeturon at 0.02 kg ha⁻¹ | - |
| 06   | Ethephon at 0.33 L ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 07   | MTD at 1.00 L ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 08   | Methyl-sulfumeturon at 0.02 kg ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 09   | Control | Water |
| 10   | Ethephon at 0.66 L ha⁻¹ | SP80-3280 |
| 11   | Ethyl-trinexapac at 0.80 L ha⁻¹ ** | - |
| 12   | Glyphosate at 0.35 L ha⁻¹ | - |
| 13   | MTD at 1.00 L ha⁻¹ | - |
| 14   | Methyl-sulfumeturon at 0.02 kg ha⁻¹ | - |
| 15   | Ethephon at 0.33 L ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 16   | MTD at 1.00 L ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 17   | Methyl-sulfumeturon at 0.02 kg ha⁻¹ | Glyphosate at 0.15 L ha⁻¹ |
| 18   | Control | Water |

* Doses usually employed by producers; ** Preliminary pot trials by our multidisciplinary team at the College of Agricultural and Technological Sciences, Unesp, found that ethyl-trinexapac and glyphosate were not complementary, causing symptoms of phytotoxicity few days after spraying, regardless dose. Because of this technical drawback, they were not tested in a mixture in the present study.

2.4. Soil Tillage and Planting of Varieties of Sugarcane

In late August 2015, the preparation of the soil consisted of plowing, harrowing and furrowing, conventionally. After approximately fifteen days of tillage, we improved acidity and fertility with the mechanical application of dolomitic limestone at 2.0 tons per hectare and NPK fertilizer at 0.6 ton per hectare, respectively. In mid-November 2015, we carried out the planting of 0.15 m length seedlings of the varieties of sugarcane into narrow furrows of 0.25 m depth, distributing them at the population density of 15 units per meter; buds were from the first ratoon season of plantations grown in the Santo Antonio Farm. During the periods of summer and winter running from December 2015 to June 2016, chemical control of sugarcane borer (Diatraea saccharalis) was performed with spray applications of the systemic insecticide, bifenthrin, at 1.2 L per hectare on the whole stem to avoid population outbreaks of the herbivory pest.

2.5. Spraying of Chemical Ripening Agents on the Varieties of Sugarcane

In early September 2016, ripeners treatments consisted of spraying the ingredients exogenously on the plants of sugarcane before the flowering stage, with T-shaped, 2 m-length, CO₂-pressurized backpack sprayer containing a set of six AXI-11002 flat spray nozzles at 0.5 m apart from one another. We performed the spraying at the pressure of 40 psi and spray flow of 300 L per hectare [6]. During the spray application in the morning, from 8:00 a.m. to 10:00 a.m., temperature and relative humidity of the air were in the ranges of 25–30 °C and 50–60%, respectively.

2.6. Technical Assessment of Antioxidant Enzymes Activities

To examine the activity of the antioxidant enzymes, ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), in the varieties of sugarcane, following the spray application of the plant growth regulators, we randomly harvested the plants from the central rows of experimental plots. In the laboratory, the pre-treatment of plant material consisted of milling the
samples of leaves in the presence of liquid-state nitrogen, followed by cooling at room temperature. Next, we carried out the homogenization of powdered plant material in a solution of 100 mM potassium buffer at pH 7.5, with 1 mM EDTA, 3 mM DTT, and PVPP at 4%. We formally centrifugated the buffered extract at 10,732.5 g per 30 min at 30 °C, then, stocked the supernatant in a life-sized stainless cooler at −80 °C to prevent it from eventual negative impacts of surrounding weather agents which might influence further analytical procedures.

2.6.1. Assay of Ascorbate Peroxidase Activity

The assay of activity of APX (EC: 1.11.1.11) consisted of monitoring spectrophotometrically the oxidation of ascorbate at 290 nm and 30 °C. The protocol of controlled reaction comprised the introduction of 40 µL plant extract into 1 mL standard medium consisting of 50 mM potassium phosphate buffer at pH 7, 0.5 mM ascorbate, 0.1 mM EDTA and 0.1 mM H₂O₂. The calculation of activity of APX, expressed as nmol ascorbate min⁻¹ mg⁻¹ protein, included the extinction coefficient, 2.8 mM⁻¹ cm⁻¹, reported by Nakano and Asada [26].

2.6.2. Assay of Catalase Activity

The assay of activity of CAT (EC: 1.11.1.6) comprised the spectrophotometry of 15 µL plant extract at 25 °C. The reaction medium contained 1 mL of 100 mM potassium phosphate buffer at pH 7.5 and 2.5 µL H₂O₂ at 30%. The determination of the activity of CAT, expressed as µmol H₂O₂ min⁻¹ mg⁻¹ protein, consisted of monitoring the removal of hydrogen peroxide at 240 nm over 1 min against plant extract-free sample, according to Gomes-Junior et al. [27].

2.6.3. Assay of Peroxidase Activity

The assay of activity of POD (EC: 1.11.1.7) consisted of monitoring the consumption of hydrogen peroxide in UV-Vis spectrophotometer at 240 nm. The protocol included the homogenization of plant extract with 1 mL 50 mM potassium phosphate buffer at pH 8.0 and further centrifugation at 15,000×g per 10 min and 4 °C. The determination of the activity of POD, expressed as nmol H₂O₂ min⁻¹ mg⁻¹ protein, followed the method of Nakano and Asada [26].

2.6.4. Assay of Superoxide Dismutase Activity

The assay of activity of SOD consisted of carrying out the electrophoresis-assisted staining of plant extract under a non-denaturing condition in 10% polyacrylamide gel, with 60 µg protein, according to Vitória et al. [28] and Gratão et al. [29]. We expressed the activity of SOD as U mg⁻¹ protein; U symbolizes the amount of SOD required to reduce H₂O₂ content by half.

2.7. Data Analysis

For the analysis of the data set, we formally ran the procedure of Shapiro-Wilk to test its normality. The significance of the effects of chemical ripening agents and varieties of sugarcane on the activity of antioxidant enzymes was tested by one-way analysis of variance (ANOVA). The mean values of the treatments were compared by post-hoc Tukey’s HSD test. Finally, we carried out the Pearson correlation test to measure the strength and direction of linear relationships between enzymes. The software was R for statistical computing and graphics.

3. Results

3.1. Effects of Chemical Ripening Agents and Varieties of Sugarcane on the Activity of Antioxidant Enzymes

Chemical ripening agent and variety of sugarcane, showed a significant interaction effect on the activity of APX, CAT, and POD (Table 3). SOD was, in turn, significantly influenced only by plant growth regulators, regardless of nature and dose of spraying solution; hence, it was not dependent on the genotypes, SP80-1842 and SP80-3280.
Table 3. Analysis of variance for the effects of chemical ripening agents and varieties of sugarcane on the activity of antioxidant enzymes.

| Source of Variation                        | Enzyme | APX | CAT | POD | SOD |
|-------------------------------------------|--------|-----|-----|-----|-----|
|                                           | F-Value|     |     |     |     |
| Chemical ripener                          | 5.66 * | 10.95 * | 22.63 * | 3.01 * |
| Variety of sugarcane                      | 0.46   | 3.95 * | 19.50 * | 0.89 |
| Chemical ripener versus variety of sugarcane | 1.93 * | 3.02 * | 12.08 * | 0.85 |
| p-value, Shapiro-Wilk test                | 0.23 * | 0.17 * | 0.66 * | 0.95 * |
| Coefficient of variation (%)              | 40.41 | 38.71 | 21.64 | 38.71 |

* Significant by Shapiro-Wilk and Fisher tests at \( p < 0.05 \); Ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD).

3.2. Activity of Antioxidant Enzymes in Varieties of Sugarcane Crop with Chemical Ripeners

3.2.1. Ascorbate Peroxidase

Ethephon plus glyphosate yielded the highest absolute value for the activity of APX in the variety, SP80-3280 (Table 4). Yet, this treatment did not differ statistically to the reference, ethephon, and glyphosate, each alone. Spraying methyl-sulfumeturon, both alone and mixed with glyphosate, on the variety, SP80-1842, caused the highest activity of this enzyme compared to the remaining compositions of chemical ripening agents. Some of the mixtures of ingredients declined, but not substantially, rate of oxidation of ascorbate by APX in both the commercial varieties of sugarcane.

Table 4. Interaction effect of chemical ripening agents and varieties of sugarcane on the activity of ascorbate peroxidase.

| Chemical Ripener | Variety of Sugarcane Crop | SP80-1842 | Mean | SP80-3280 | Mean | SP80-1842 Mean | F-Value |
|------------------|---------------------------|-----------|------|-----------|------|----------------|---------|
| Ethephon         | 1.35 aA                   | 1.15      |      | 1.125     | 1.25 | 10.80 *        |         |
| Ethyl-trinexapac | 0.70 aA                   | 0.725     |      | 1.05      | 0.75 | 7.80 *         |         |
| Glyphosate       | 0.69 aA                   | 0.675     |      | 0.725     | 0.725|               |         |
| MTD              | 0.75 aA                   | 0.75 aA   |      | 0.40      |      |                |         |
| Methyl-sulfumeturon | 1.45 aA               | 0.80 ab |      | 1.125     | 1.25 | 11.50 *        |         |
| Ethephon plus glyphosate | 1.70 aA      | 1.45      |      | 1.25      | 1.25 | 11.50 *        |         |
| MTD plus glyphosate | 1.20 aA       | 1.20 ab  |      | 1.05      | 0.75 | 7.80 *         |         |
| Methyl-sulfumeturon plus glyphosate | 0.90 aA       | 0.90 ab |      | 1.25      | 1.25 | 11.50 *        |         |
| Control          | 1.15 aA                   | 1.125     |      | 0.05      |      |                |         |
| Mean             | 1.05                      | 0.90      |      |           |      |                |         |
| F-value          | 1.90                      | 2.25      |      |           |      |                |         |

Mean values superscripted by the same capital letters in lines and lowercase letters in columns do not differ by post-hoc Tukey’s HSD test at \( p < 0.05 \); * Significant by Fisher test at \( p < 0.05 \).

3.2.2. Catalase

The mixture of MTD with glyphosate triggered the highest activity of CAT in both the varieties of sugarcane, but SP80-1842 and SP80-3280 differed statistically in the performance of this enzyme (Table 5). The chemical treatment of SP80-1842 and SP80-3280 with this combination of ripening ingredients tightly increased the rate of oxidation of \( \text{H}_2\text{O}_2 \) by CAT by approximately 130% and 60%, respectively, in comparison to controls. Conversely, spraying the ethephon, ethyl-trinexapac, glyphosate, MTD and methyl-sulfumeturon, alone, slightly declined the activity of CAT in SP80-3280 compared to control. The variety, SP80-1842, interestingly had a higher activity of CAT, compared to SP80-3280, in the presence of ethephon, ethyl-trinexapac, glyphosate, methyl-sulfumeturon and methyl-sulfumeturon with glyphosate.
Table 5. Interaction effect of chemical ripening agents and varieties of sugarcane on the activity of catalase.

| Chemical Ripener       | Variety of Sugarcane Crop | Mean       | F-Value |
|------------------------|---------------------------|------------|---------|
|                        | SP80-1842                 | SP80-3280  |         |
|                        | Activity of CAT (µmol H₂O₂ min⁻¹ mg⁻¹ Protein) |           |         |
| Ethephon               | 0.20 bA                   | 0.15 bB    | 0.175   | 8.70 *  |
| Ethyl-trinexapac       | 0.35 bA                   | 0.20 bB    | 0.275   | 9.80 *  |
| Glyphosate             | 0.25 bA                   | 0.20 bB    | 0.20    | 13.55 * |
| MTD                    | 0.20 bA                   | 0.20 bA    | 0.20    | 1.40    |
| Methyl-sulfumeturon    | 0.25 bA                   | 0.20 bB    | 0.225   | 14.10 * |
| Ethephon plus glyphosate| 0.40 bA                  | 0.40 bA    | 0.40    | 0.30    |
| MTD plus glyphosate    | 0.65 aA                   | 0.45 ab    | 0.55    | 10.85 * |
| Methyl-sulfumeturon plus glyphosate | 0.30 bA | 0.25 abA | 0.275   | 2.15    |
| Control                | 0.30 bA                   | 0.25 abB   | 0.275   | 15.30 * |
| Mean                   | 0.30                      | 0.25       | 0.275   |         |
| F-value                | 6.20 *                    | 3.70 *     |         |

Mean values superscripted by the same capital letters in lines and lowercase letters in columns do not differ by post-hoc Tukey’s HSD test at 0.95 confidence level; * Significant by Fisher test at 0.95 confidence level.

3.2.3. Peroxidase

Ethephon was the most technically efficient ripening ingredient in inducing the activity of POD in the variety, SP80-1842, followed by glyphosate and MTD both, alone and methyl-sulfumeturon and glyphosate in a mixture (Table 6). The ethylene-releasing product, the broad-spectrum non-selective herbicide, and the insecticide/acaricide impressively increased the rate of oxidation of H₂O₂ by POD by 100%, 50%, and 50%, respectively compared to the control. These treatments did not differ statistically. Regarding the variety, SP80-3280, all plant growth regulators significantly declined the activity of POD, as compared to the reference. In the absence of chemical ripeners, the activity of this antioxidant enzyme was significantly higher in SP80-3280 than that in SP80-1842.

Table 6. Interaction effect of chemical ripening agents and varieties of sugarcane on the activity of peroxidase.

| Chemical Ripener       | Variety of Sugarcane Crop | Mean       | F-Value |
|------------------------|---------------------------|------------|---------|
|                        | SP80-1842                 | SP80-3280  |         |
|                        | Activity of POD (nmol H₂O₂ min⁻¹ mg⁻¹ Protein) |           |         |
| Ethephon               | 0.20 aA                   | 0.05 cB    | 0.125   | 37.70 * |
| Ethyl-trinexapac       | 0.10 bca                   | 0.10 bA    | 0.10    | 2.60    |
| Glyphosate             | 0.15 ba                   | 0.10 bca   | 0.125   | 7.30 *  |
| MTD                    | 0.15 ba                   | 0.05 cb    | 0.10    | 15.90 * |
| Methyl-sulfumeturon    | 0.05 da                   | 0.10 bca   | 0.075   | 3.15    |
| Ethephon plus glyphosate| 0.05 db                  | 0.10 ba    | 0.075   | 6.20 *  |
| MTD plus glyphosate    | 0.10 ca                   | 0.10 bA    | 0.10    | 1.40    |
| Methyl-sulfumeturon plus glyphosate | 0.15 ba | 0.05 db | 0.10    | 11.40 * |
| Control                | 0.10 bcA                  | 0.15 bA    | 0.125   | 16.50 * |
| Mean                   | 0.10                      | 0.10       |         |         |
| F-value                | 20.90 *                   | 15.60 *    |         |

Mean values superscripted by the same capital letters in lines and lowercase letters in columns do not differ by post-hoc Tukey’s HSD test at 0.95 confidence level; * Significant by Fisher test at 0.95 confidence level.

3.2.4. Superoxide Dismutase

Ethyl-trinexapac, glyphosate, MTD, and methyl-sulfumeturon had a positive effect on the activity of SOD, but they did not differ statistically (Table 7). In particular, the broad-spectrum non-selective herbicide substantially increased the rate of oxidation of H₂O₂ by SOD by approximately 55%, compared to the reference. Ethephon, both alone and mixed with glyphosate, had similar performance compared to control. The mixture of ethephon plus glyphosate was the only inefficient to increase the activity of
SOD in the varieties of sugarcane. On the contrary, this spraying solution reduced, but not significantly, enzymatic scavenging of H$_2$O$_2$ by SOD. SP80-1842 and SP80-3280 had statistically similar absolute values for the activity of this antioxidant enzyme.

**Table 7.** Effects of chemical ripening agents and varieties of sugarcane on the activity of superoxide dismutase.

| Chemical Ripening Agent                  | Activity of SOD (U mg$^{-1}$ Protein) |
|------------------------------------------|--------------------------------------|
| Ethephon                                 | 100.50$^{A}$                         |
| Ethyl-trinexapac                         | 135.25$^{A}$                         |
| Glyphosate                               | 152.10$^{A}$                         |
| MTD                                      | 130.05$^{A}$                         |
| Methyl-sulfometuron                      | 137.15$^{A}$                         |
| Ethephon plus glyphosate                 | 90.75$^{B}$                          |
| MTD plus glyphosate                      | 135.10$^{A}$                         |
| Methyl-sulfometuron plus glyphosate      | 125.75$^{A}$                         |
| Control                                  | 97.90$^{B}$                          |
| Variety of sugarcane crop               |                                       |
| SP80-1842                                | 135.10$^{A}$                         |
| SP80-3280                                | 135.05$^{A}$                         |

Mean values superscripted by the same capital and lowercase letters do not differ by post-hoc Tukey’s HSD test at $p < 0.05$.

3.2.5. Linear Associations between Antioxidant Enzymes

Briefly, catalase negatively correlated with the superoxide dismutase (Table 8).

**Table 8.** Linear relationships between antioxidant enzymes in the popular varieties of sugarcane, SP80-1842 and SP80-3280, with spray application of chemical ripening agents.

|                        | CAT  | APX  | SOD  |
|------------------------|------|------|------|
| Ascorbate peroxidase   | 0.30 |      |      |
| Superoxide dismutase   | −0.60$^{*}$ | −0.30 |      |
| Peroxidase             | 0.20 | −0.15 | −0.20 |

$^{*}$ Significant by Pearson test at $p < 0.05$.

4. Discussion

Superoxide dismutase is one of the most effective antioxidant enzymes to alleviate the negative effects of ROS on crop plants under stressing environments like drylands [18,30,31]. Gomathi and Rakkiyapan [12] reported that SOD significantly reduced the lipid peroxidation and degradation of the cellular membrane by water stress by flooding and salinity in the commercial varieties of sugarcane, CO 8371, CO 86032, CO 99004 and CO 99006. Boaretto et al. [3] also emphasized the great potential of SOD to mitigate oxidative stress by drought in the Brazilian popular varieties, IACSP 95-5000 and IACSP 94-1094. The authors argued this antioxidant enzyme was efficient in preserving the dehydration of photosynthetic and non-photosynthetic tissues of plants in low water content substrate. Contextually, Jangpromma et al. [32] endorsed the benefits of SOD to chlorophyll content and photosynthetic performance of sugarcane stressed by drought. The notable enhancement of the activity of SOD by spray application of ethyl-trinexapac, glyphosate, MTD and methyl-sulfumeturon, either alone or in a mixture, could, therefore, help the sugar-energy sector to produce suitable feedstock for the fabrication of sugar and biofuels in microclimates which either drought or salinity limits the plant growth and development.

Most of the spraying solutions, containing glyphosate, were highly effective in increasing the rate of oxidation of ascorbate and hydrogen peroxide by APX, CAT, POD, and SOD. Ashad et al. [33] asserted the glyphosate at balanced endogenous levels improved the enzymatic performance of APX, glutathione reductase (GTS), nucleoside diphosphate kinase (NDPK), thioredoxin and SOD by exciting
the electron flow into cellular compartments. Percival [34] incisively stated that glyphosate, at low doses, increased the concentrations of the non-enzymatic and enzymatic antioxidants, carotenoids, proline, CAT and SOD, due to the hormesis effect. The references were in line with the enhanced activity of APX and CAT in the varieties of sugarcane, SP80-1842 and SP80-3280, with MTD and ethephon, and both mixed with glyphosate at half of the dose. In the plant, glyphosate primarily acts as an inhibitor of the enzyme, 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS), which is the core of shikimic acid’s pathway. Shikimate is one of the most relevant precursing metabolites of aromatic amino acids (e.g., phenylalanine, tryptophan and tyrosine), auxins, salicylic acid, and flavonoids. The inhibition of EPSPS by glyphosate triggers events of oxidative stress, mainly including lipid peroxidation, degradation of phytoalexins and depigmentation of photosynthetic parts of the plant. In response to induced oxidative stress, the plant synthesizes antioxidants to fight free radical [35–37]. Thus, glyphosate probably increased the activity of APX, CAT, POD, and SOD in plants of sugarcane by chemically stressing the pathway of shikimate.

Gill and Tuteja [38] found the antioxidant enzyme, CAT, was highly effective in detoxifying H$_2$O$_2$ in plants of sugarcane under oxidative stress through extreme temperatures. The mixture of MTD with glyphosate, both at half the doses, could eventually alleviate the harmful effects of oxidation by thermal stress on sugarcane crop, as it significantly increased the activity of CAT in the varieties, SP80-1842 and SP80-3280. Few scientific studies report the great potential of the chlorophenols and fluroxypyr at low doses in enhancing the consumption of hydrogen peroxide and superoxide by catalase in crops [39,40]. Apart from herbicides, ethylene also alters the dynamics of antioxidant enzymes by inducing H$_2$O$_2$-transcribing genes [41]. To preserve hydrogen peroxide at harmless levels to cellular growth and development, the plant produces ROS-scavenging enzymes, such as catalase. Sakamoto, Munemura, and Tomita [42] stated the ethylene, at balanced levels, increased the activity of APX, CAT, SOD, and glutathione reductase, as well as the concentration of proline. The authors still adverted the plant growth regulator at unbalanced levels made ROS exceed the redox potential of the plant stress defense system. The reference was in agreement with the lowered activity of POD in the variety of sugarcane, SP80-3280, with ethylene-releasing ethephon, both alone and mixed with glyphosate. This antioxidant enzyme was also highly sensitive to MTD and methyl-sulfumeturon. Indeed, the activity of ROS-scavenging enzymes depended on the nature and dose of spraying solution of ripening chemicals. Misusing plant growth regulators could, therefore, be potentially risky to the antioxidant enzymatic system of sugarcane crop, making it vulnerable to stressful factors. In addition to fighting free radical, APX, CAT, POD, and SOD also play a key role in physiological ripening in sugarcane crop, according to Vasantha et al. [43].

Scientific studies on the antioxidant mechanisms in crop plants, under stressing conditions, report that APX and CAT act in secondary detoxification of ROS mainly by transforming the hydrogen peroxide into H$_2$O. Water is the product of enzymatic degradation of either, O$_2^-$ or OH$^-$ by SOD, which performs primary detoxication of free radicals [44,45]. The references were in line with the negative correlation between CAT and SOD. The negative linear relationship between CAT and SOD meant that the higher the activity of superoxide dismutase in earlier stages of detoxification, the lower the activity of catalase in later stages, as the lower the availability of H$_2$O$_2$ for enzymatic conversion into non-reactive oxygen species. Ascorbate peroxidase positively correlated with the resistance of plant crops to stressful agents, as pointed out by Gomathi, Manobari, and Rakkiyapan [46]. Therefore, enhanced activity of APX in plants of sugarcane with chemical ripeners, especially methyl-sulfumeturon mixed with glyphosate at half of the dose, could collaborate with CAT and SOD to successfully alleviate oxidation by stressful agents in plantations in harsh climates. Irrespective of the composition of spraying solution, glyphosate at half of the dose was the most persistent synthetic product to make APX, CAT, and SOD more efficient in consuming the reactive oxygen species in the Brazilian popular varieties, SP80-1842 and SP80-3250, under chemically induced oxidative stress. Chahid et al. [23] reported the antioxidant potential of APX tightly decreased, as the dose of insecticide increased. The reference was consistent with the lowered activity of APX in sugarcane plants with MTD, both alone.
and mixed with glyphosate. Thus, the dose of this insecticide/acaricide must be reduced, since spraying it at 1.00 L per hectare proved to be limiting for the ascorbate peroxidase.

Chemical ripening by ethephon improved the activity of POD in plants of sugarcane and maize under thermal and hydric stress [47,48]. The references supported the enhanced activity of POD in the variety, SP80-1842, with ethephon. The decreased activity of POD in the variety, SP80-3280, with the application of the same chemical ripening agent, proved the oxidation of hydrogen peroxide by POD was also dependent on the genotype. The lowered activity of POD in the variety, SP80-1842, with ethephon in mixture with glyphosate, meant these active ingredients at low doses were not synergistic to each other, in order to enhance the enzymatic performance of peroxidase. MTD and methyl-sulfumeturon with glyphosate were other antagonistic combinations of chemical ripeners for the activity of POD in the variety, SP80-3280. Physicochemical properties and doses of ethephon, glyphosate, MTD and methyl-sulfumeturon influenced the effectiveness of mixtures to enhance the activity of APX, CAT, POD, and SOD in plants of sugarcane. Globally, our discoveries on the activity of APX, CAT, POD, and SOD in the popular varieties, SP80-1842 and SP80-3280, with chemical ripeners, proved the practicability and efficiency of ethephon, ethyl-trinexapac, glyphosate, MTD, and methyl-sulfumeturon in enhancing the reactivity of enzymatic antioxidant system in plants of sugarcane. The information may be useful to those producers who may be interested in using these ingredients as a strategy to manage this multi-purpose energy-crop in marginal zones.

5. Conclusions

Spraying solutions consisting of glyphosate at half of the dose both with the insecticide/acaricide, MTD and with the ethylene-releasing product, ethephon, are the most technically effective pathways to enhance the reactivity of antioxidant enzymes to free radical; they could, therefore, enable sugarcane crop to perform adequately under uncontrollable or unpredictable agroecosystems for natural ripening in marginal zones, where drought, heat, salinity, and pests make planning of sugar-energy sector difficult and reduce productivity and the quality of feedstock for the production of sugar and biofuels, as well as for the cogeneration of heat and bioelectricity.

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