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Oxidative enzymes activity in sugarcane juice as a function of the planting system

Atividade de enzimas oxidativas em caldo de cana-de-açúcar em função do sistema de plantio

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Abstract

In Brazil, the largest producer of sugarcane in the world, the industrial process transforms this crop into ethanol and/or granulated sugar. Some cultivars exhibit enzymatic browning in the extracted sugarcane juice at levels harmful to the manufacturing process of white granulated sugar. The objective of this study was to assess the effect of sugarcane straw used as soil coverage, the use of different planting systems, and treatments with hydrogel polymer on enzymatic activity. The cultivar RB 86 7515 was sampled for 8 months; the first sample was obtained by cutting the upper portion of the stalk at the internode, which was taken to the laboratory for determination of the enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD). The soil coverage with different forms of straw as well as the planting systems did not change the enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD). The polyphenoloxidase (PPO) activity increased with the use of a polymer due to increased polyphenoloxidase (PPO) activity in the groove system. The enzymes studied showed changes in activity during the experimental period. The production of sugar at the end of the season (August to November) avoids the periods of highest enzymatic activity.
Keywords: *Saccharum; straw coverage; enzymes.*

Resumo

No Brasil, o maior produtor de cana-de-açúcar do mundo, o processamento industrial transforma a cana-de-açúcar principalmente em etanol e/ou açúcar cristal. Alguns cultivares apresentam escurecimento enzimático no caldo extraído em níveis prejudiciais ao processo de fabricação do açúcar cristal branco. O objetivo da pesquisa foi avaliar o efeito do palhiço como cobertura, sistemas de plantio e tratamentos com polímero hidrogel em atividades enzimáticas. O cultivar RB 86 7515 foi amostrado por oito meses, retirando-se o primeiro entrenó da parte superior, que foi levado ao laboratório e procedeu-se à determinação das atividades da polifenoloxidase (PFO) e da peroxidase (POD). As coberturas do solo com diferentes formas de palhiço, bem como os sistemas de plantio, não promoveram alterações nas atividades enzimáticas das enzimas polifenoloxidase (PFO) e peroxidase (POD). A atividade da PFO foi incrementada com a utilização de polímero, este fato foi promovido pelo aumento de atividade da PFO no sistema de plantio em sulco. As enzimas estudadas apresentaram mudanças de atividades nos meses. A produção de açúcar no final da safra (agosto a novembro) evita os períodos de maiores atividades enzimáticas.
Palavras-chave: *Saccharum; cobertura; enzimas.*

1 Introduction

In the first decade of the 21st century, sugarcane production and cultivated areas grew as a result of the demand for renewable fuels, such as ethanol from sugarcane, and the international market demand for sugar, facts that contributed to price increase of this commodity (SANTOS; BORÉM; CALDAS, 2011).

The increases in production led to diverting land used for cattle ranching (low level of production) to the production of sugarcane.

Brazil is the world's largest producer of sugarcane, with 8.43 million hectares and an estimated production of 588.9 million tons in 2012, which was 5.64% lower than that of the previous harvest (2009/2010) (623.9 million tons). Brazil's Center-South region is responsible for 88.18% of the harvest in the country, and in the State of São Paulo it was estimated a production of 320.6 million tons in an area of 4.4 million hectares (COMPANHIA..., 2011).

Physical and chemical treatments are necessary in the manufacturing process of granulated sugar from sugarcane aiming to clarify the broth and produce clearer sugars, which have greater value in the market. The dark pigments in plants can be of non-enzymatic or enzymatic-origin; the latter results from the activities of oxidases present in the industrial process (REIN, 2007).

Soil moisture and temperature promotes changes in the biometrics of sugarcane by increasing the number of tillers, thus increasing the average height of the stems, and it can promote changes in sugarcane quality and quantity (BONNETT, 1998; SINGELS et al., 2005; SINGH; SHUKLA; BHATNAGAR, 2007; ALMEIDA et al., 2008).

This research hypothesis is that using different doses of hydrogel polymers, different amounts of dry matter as cover, and systems with different planting depths interferes with the

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physiology of plant growth promoting changes in enzyme activities. The objective of this study was to assess the effect of sugarcane straw used as soil coverage, the use of different planting systems, and treatments with hydrogel polymer on enzymatic activity.

2 Materials and methods

The study was conducted in the experimental area of the University of the Western São Paulo (Unoeste), campus II, in Presidente Prudente-SP, latitude 22° 07' 04" South, longitude 51° 22' 04" West at 430 m above sea level. The cultivar of sugarcane RB 867515 was planted in December 2007 and harvested in June 2009 (18 months). After harvest the first sugarcane ratoon was used in this study. The soil was identified as Red-Yellow Argisols (EMBRAPA, 1999), a type C production environment (PRADO, 2005). According to Koppen, the climate of the region is classified as Aw. The weather data such as rainfall and maximum and minimum temperature were collected during the experiment period. A composite soil sampling was performed 60 days preceding planting and soon after sugarcane harvest.

According to the recommendations of Espironelo (1992), limestone addition was not necessary to correct the soil acidity, but it was fertilized (2007 harvest season) with the equivalent of 0 kg ha^{-1} of N, 135 kg ha^{-1} of P_2O_5, and 135 kg ha^{-1} of K_2O using 675 kg ha^{-1} of 20-20-00 fertilizer. In February 2008, the plants received 30.8 kg ha^{-1} N in the form of 70 kg ha^{-1} urea (44%N) when the average plant height was 0.20 m,

A complete randomized block design in plot subdivided was used: block 1- planting groove system and block 2 – windrow system. Between each block, four doses of water-absorbing polymer were tested. In these plots, the doses were subdivided into four treatments using different amounts of dry matter of sugarcane straw as coverage (2 × 4 × 4). The enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD) was measured for eight months; apical meristem samples were collected, placed in a cooler and forwarded to the laboratory, and pressed at 250 kgf cm^{-2}. The juice obtained (150 g) was prepared according to Vanini, Kwiastokowski and Clemente (2010)

The experimental unit (sub-plot) consisted of five rows of 5 meters long and spacing of 1.5 m (area of 37.5 m^2). Culms were collected monthly from March to October 2010 for juice extraction and determination of enzymatic activity, according to Campos et al. (1996). Sugarcane samples were collected in three rows (3 repetitions) excluding the first and the last microplot of 1 m in each row. The first culms, below the insertion of the TVD (Top Visible Dewlap), were collected and pressed at the laboratory. The sugarcane juice extracted was quickly analyzed.

The amounts of dry matter used were of 0 t ha^{-1}, 5 t ha^{-1}, 10 t ha^{-1}, 15 t ha^{-1} used the humidity 40% on green leaves according Orlando Filho (1983), Bovi and Serra (2001) and Ripoli and Ripoli (2004). These treatments with the addition of straw started after the first harvest (June of 2009). The data were submitted to analysis of variance by the F-test at 5% probability level. The means were compared by the Scott-Knott test using the SISVAR statistical software (ANOVA, $p < 0.05$), according to Gomes (1990). For the regression analysis of enzymatic activity data, the Microcal Origin 6.0 software was used.

3 Results and discussion

The weather data of the rainfall, maximum, and minimum temperatures collected are shown in Figure 1. Table 1 presents the results of soil analysis carried out on the experimental soil 60 days preceding planting and soon after the harvest of sugarcane.

During the experimental period (March to October), the synthetic polymer promoted changes in the PPO activity (Table 2).

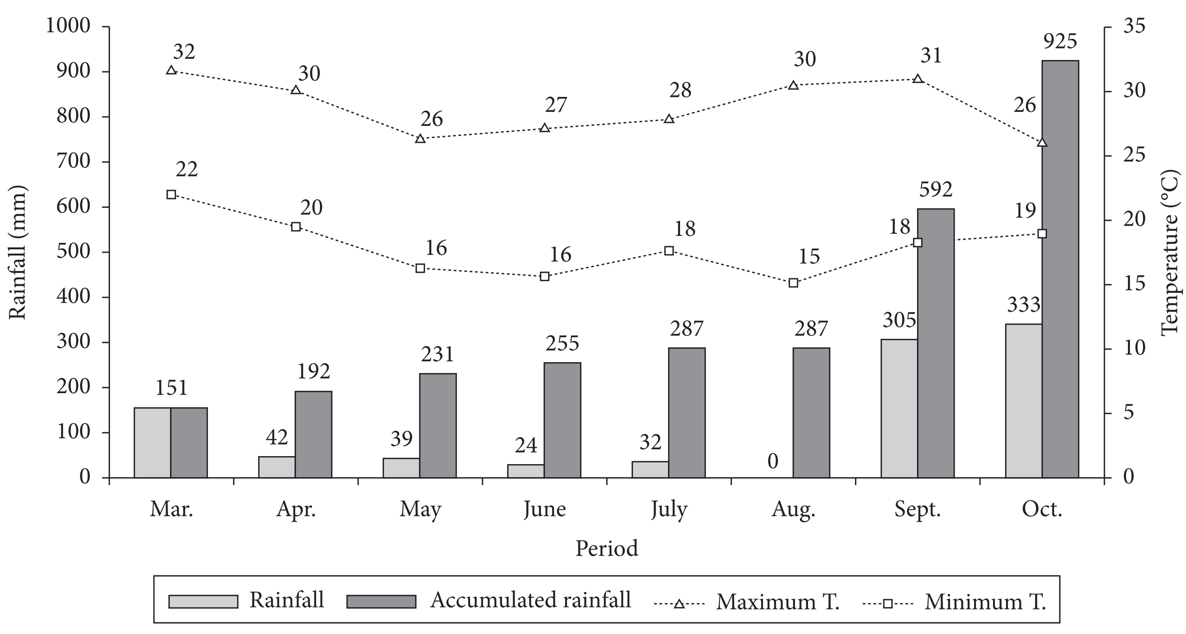

Figure 1. Weather information obtained at the meteorological station of Unoeste during the experimental period in 2010.

Table 1. Analysis of the experimental soil before planting and after harvest of sugarcane.

			October 2007			
pH in $CaCl_2$	pH in SMP	Pot. Acidity (H+AL) (mmolc/dm^3)	Aluminum($Al+^3$) (mmolc/dm^3)	Org. Mat. (g/dm^3)	Calcium($Ca+^2$) (mmolc/dm^3)	Magnesium($Mg+^2$) (mmolc/dm^3)
5.5 (Medium)	7.3	11	0	5 (Sandy soil)	11 (High)	6 (Medium)
Potassium(K+) (mmolc/dm^3)	Phosphorus (mg/dm^3)	Sulphur(SO^2) (mg/dm^3)	exchangeable K, Ca, and Mg (mmol/dm^3)	Al^{+3} saturation (%)	CEC (mmolc/dm^3)	B.S(%)
1.4 (Bass)	21 (Medium)	1.3 (Bass)	18	0	29	63 (Medium)
			July 2009			
pH in CaCl	pH in SMP	Pot. Acidity (H+AL) (mmolc/dm^3)	Aluminum($Al+^3$) (mmolc/dm^3)	Org. Mat. (g/dm^3)	Calcium($Ca+^2$) (mmolc/dm^3)	Magnesium($Mg+^2$) (mmolc/dm^3)
5.5	7.1	14.2	0	7.4 (Sandy soil)	8.2 (High)	3.4
Potassium(K+) (mmolc/dm^3)	Phosphorus (mg/dm^3)	Sulphur (SO^{-2}) (mg/dm^3)	exchangeable K, Ca, and Mg (mmol/dm^3)	Al^{+3} saturation (%)	CEC (mmolc/dm^3)	B.S(%)
2.0 (Medium)	13.9 (Bass)	1.3 (Bass)	13.7	0	27.8	48.8 (Bass)

Table 2. Enzymatic activity (au - activity unit) of PPO and POD in the planting systems and polymer doses

Quantities (kg ha^{-1})	Windrow	Groove	Average
		PPO	
0.00	210^{Ba}	200^{Ba}	200^{B}
26.66	300^{Aa}	200^{Bb}	250^{A}
53.33	240^{Ba}	260^{Aa}	250^{A}
80.00	220^{Ba}	240^{Aa}	230^{A}
Average	240^{a}	220^{a}	230
		POD	
0.00	760^{Aa}	740^{Aa}	750^{A}
26.66	730^{Aa}	530^{Ba}	630^{A}
53.33	620^{Aa}	790^{Aa}	700^{A}
80.00	660^{Aa}	820^{Aa}	740^{A}
Average	690^{a}	720^{a}	700

Uppercase letters in the same column and lowercase letters in the same row denote significant difference ($p < 0.05$) by the Scott-Knott.

Table 3. Enzymatic activity (au - activity unit) of PPO and POD in the planting systems and coverage (DM – dry matter).

Coverage (t DM ha^{-1})	Windrow	Groove	Average
		PPO	
0	230^{Aa}	240^{Aa}	230^{A}
5	280^{Aa}	220^{Ab}	250^{A}
10	240^{Aa}	220^{Aa}	230^{A}
15	240^{Aa}	220^{Aa}	230^{A}
Average	240^{a}	220^{a}	230
		POD	
0	722^{Aa}	726^{Aa}	724^{A}
5	567^{Aa}	646^{Aa}	606^{A}
10	729^{Aa}	726^{Aa}	728^{A}
15	732^{Aa}	774^{Aa}	753^{A}
Average	687^{a}	718^{a}	703

Uppercase letters in the same column and lowercase letters in the same row denote significant difference ($p < 0.05$) by the Scott-Knott.

According to Bucheli and Robinson (1994), PPO is the enzyme with the highest activity in sugarcane juice, and Willadino et al. (2011) reported that this enzyme is related to unfavorable response mechanism factors such as water stress and can provide changes in the metabolism of plants (ZHU, 2002; FOYER; NOCTOR, 2005; CAVALCANTI et al., 2004). Some authors have observed increased PPO activity at the end of the sugarcane season (MOURA et al., 1999; BRITO et al., 2007; AZEVEDO et al., 2009). One can infer that the use of polymers changes the metabolism of plants due to the better availability of water, a fact that was detected by increasing the enzymatic activity of PPO.

No significant changes were observed in the enzymatic activity of POD (Table 2). On average, there was an increase in the enzymatic activity of PPO with the use of the polymers.

As can be seen in Table 3, the use of different doses of coverage did not promote changes in the enzymatic activity of PPO and POD. This fact indicates that the plants had a weak interaction with the use of coverage, which is usually used to improve soil physical and chemical attributes such as water holding capacity and the provision of water for cultivated plants. Therefore, the polymer used, which promoted changes in the enzymatic activity, showed a stronger plant-soil relationship.

It can be observed in Figure 2 that there was a quadratic regression between the months and the PPO enzyme activities with a significant statistical adjustment at the level of 1%. Deriving the equation and equalizing to zero, the curve maximization point is obtained, which in this case is 4.48 (between April and may) with 290 au activity. This enzymatic activity plays an important role in the stress that occurs during these months. Figure 1 shows rainfall reduction, which in sandy soils, promotes lower humidity in the soil leading to greater soil water stress. This fact leads to the browning of sugarcane juice, according to Qudsieh et al. (2002) and Bucheli and Robinson

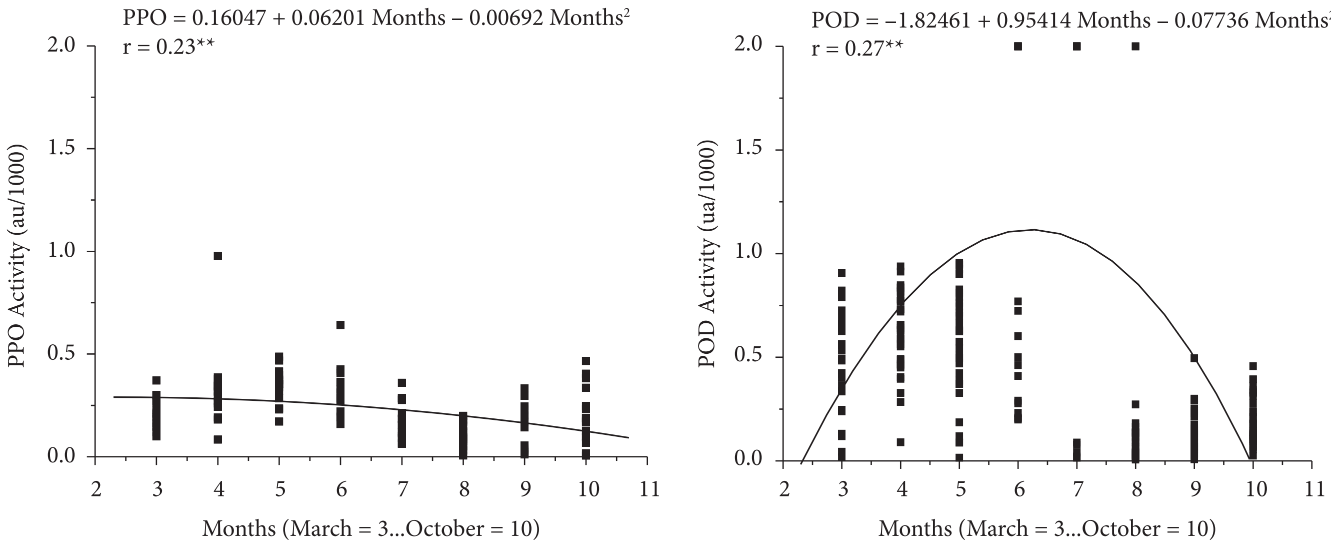

Figure 2. Enzymatic activity of PPO and POD during the study period.

(1994). For the production of whiter sugar, periods of increased enzymatic activity should be avoided, which in this study took place at the beginning of the harvest of sugarcane in the Center-South region of Brazil. In Figure 2, it can also be seen the quadratic correlation of POD activity that had its peak between June and July with the value of 1120 au; however, this enzyme has been reported to have low activity and little relationship with the browning of sugarcane juice (BUCHELI; ROBINSON, 1994).

4 Conclusions

The coverage used with different doses of straw (0, 5, 10, 15 t DM ha^{-1}) did not promote changes in the enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD) in the experimental period.

The planting systems studied (windrow and groove) did not promote changes in the enzymatic activity of polyphenoloxidase (PPO) and peroxidase (POD).

Polyphenoloxidase (PPO) activity, on average, increased with the use of the polymer.

The polyphenoloxidase (PPO) and peroxidase (POD) activity changed during the period of time evaluated.

Sugar production at the end of the season (August to November) avoids periods of highest enzymatic activity.

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