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CROP PRODUCTION

Activity invertase and amylase in Marandu grass under shading and nitrogen fertilization

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ABSTRACT. The objective of this study was to evaluate the activity of invertases and amylases in *Brachiaria brizantha* cv. Marandu under various shade and nitrogen fertilization conditions. The experiment was carried out in a greenhouse using a 4 x 2 factorial scheme (shading levels of 0, 30, 50, and 80% and fertilization with 0 and 100 kg N ha^{-1}). The activity of the enzymes, cytosol-neutral invertase (Inv-N), vacuole acid (Inv-V) and cell-acidic acid (Inv-CW), reducing sugars (RS), and α and β-amylases were evaluated (α = 0.05). The interaction was significant for Inv-N within the leaf. In the first cycle, the highest activity was in fertilized plants with 30, 50, and 80% shading. For Inv-CW in the 1st cycle, the highest activity occurred with 0, 30, and 50% shading. However, the interaction for Inv-V leaf activity was not significant in the 1st and 2nd cycles. The highest activity observed for Inv-V was in the fertilized plants, suggesting that fertilization increased the enzymatic activity. The activity of the invertases increased both under 30-50% shaded conditions and in full sun. Furthermore, invertase activity was directly linked to the osmoregulatory system. The reduction in RS was related to a low photosynthetic rate, and an increase α and β-amylase was associated with the use of reserve energy sources to meet energetic needs.

Keywords: carbohydrates; invertases; photosynthesis.

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Introduction

Brachiaria are photoautotrophs, and, as such, they depend on the acquisition of luminous energy for growth and persistence. When the plant is under threat of survival due to light limitations, evolutionary mechanisms direct highly adaptive strategies to tolerate or escape shading caused by nearby vegetation (Franklin & Whitelam, 2005). Because of this, plants are continuously adjusting their metabolism and developing strategies for growth and development to optimize the photosynthetic activity during light competition.

The mechanism of the active accumulation of sucrose is dependent on the maturity of the tissues. That is, there is a difference between mature and immature tissues, mainly due to the concentration of invertases and the need for growth (Lingle, 1999).

Degradation of sucrose, and consequently the establishment of the drain, can be accomplished by both sucrose synthase (SuSy) and invertase (Bieniawska et al., 2007). The invertase enzymes in the forages are found in several isoforms, each displaying different biochemical properties and subcellular locations: neutral invertase, vacuolar acid invertase, and acidic cell wall invertase (Tymowska-Lalanne & Kreis, 1998; Rohwer & Botha, 2001).

Thus, the constitution of the forage space encompasses both morphological and structural properties, as well as the biochemistry and physiology of the cultivated plants. The interaction of biotic and abiotic factors reflects on the responses to different stimuli, which may alter carbohydrate metabolism and the partitioning of photoassimilates at different stages of development.

The objective of this study was to evaluate the activity of invertebrates and amylases in *Brachiaria brizantha* cv. Marandu under different shade and nitrogen fertilization conditions.

Material and methods

The experiment was carried out in a greenhouse from July 1 to November 9, 2016, at the State University of Southwest of Bahia - Campus "Juvino Oliveira" in Itapetinga, Bahia State, Brazil (coordinates: 15º38'46" south latitude, 40º15'24" west longitude). The average altitude is 280 m. The climate, according to the Köppen classification, is type "Cw", mesothermic wet and warm subhumid.

The experiment was conducted in a 4 x 2 factorial scheme with four replications, with four levels of shading (0, 30, 50, and 80% shading) and with and without nitrogen fertilization of 100 kg N ha^{-1} in one randomized block design, totaling 32 plastic vessels with a capacity of 10 dm^3 and an experimental period of 84 days.

The soil used was collected at Fazenda Bela Vista, located in the municipality of Encruzilhada, Bahia State, Brazil, and was classified as dark red Latosol, with a sandy clay loam texture. For soil analysis, simple samples were collected at random points with depth 0 - 20 cm, air dried, homogenized to obtain the composite sample, then sent to the Department of Agricultural Engineering and Soils of the UESB to carry out analyzes chemical characteristics; the soil presented the following characteristics: pH = 5.1; P = 2.0 mg dm^{-3}; K = 0.30 $cmol_c$ dm^{-3}; Ca = 1.7 $cmol_c$ dm^{-3}; Mg = 1.03 $cmol_c$ dm^{-3}; Al = 0.4 $cmol_c$ dm^{-3}; base sum = 3.3 $cmol_c$ dm^{-3}; cation exchange capacity at pH 7.0 = 8.0 $cmol_c$ dm^{-3}; base saturation = 41%; and organic matter = 25 g dm^{-3}.

According to the results of the soil analysis and following the recommendations of the Soil Fertility Commission of the State of Minas Gerais (Alvarez & Ribeiro, 1999), where the technological average level was adopted, it was necessary to perform liming considering the low calcium/magnesium ratio to raise the base saturation to 50; 4.8 g per pot of calcitic limestone was applied 30 days before transplanting of the seedlings, and phosphate fertilization performed at the time of planting with 90 kg ha^{-1} of P_2O_5, equivalent to 1.3 g of triple superphosphate per vessel. Nitrogen fertilization was carried out in two installments, with a total of 100 kg of nitrogen per hectare, corresponding to 1.8 g of urea per pot.

To determine the field capacity, the vessels with dry soil were weighed and then were soaked and weighed again after the total water flow. The maximum water retention capacity was determined by the dry and wet weight difference, which was approximately 18%.

Planting was carried out on July 1, 2016 in pots for seedling production using commercially sold Capim Marandu seeds, and a total of ten seedlings for each experimental vessel were transplanted on July 15. After 15 days, thinning was done, leaving four seedlings per pot. On August 16, a uniform cut was made at a height of 10 cm from the soil, and the shading was implemented. The position of the nylon screens was east-west. The percentages of shading used were 0, 30, 50, and 80%, and shading was installed according to the manufacturer.

The plants were irrigated daily, maintaining the field capacity during the experimental period. The minimum, maximum and average temperatures and humidities were measured during the experimental period from August 16 to November 9, 2016, totaling 84 days.

The collections for the enzymatic analyzes were performed in two cycles, on September 13 and November 9, which corresponded to 28 after the cut of uniformization and 28 days after the second cut at 15 cm in height, respectively. The third or fourth fully expanded leaf, stem and root of the plants were collected at the end of the second period when the vessels were removed. At the time of collection, the plant material was placed in aluminum foil bags, previously identified and subsequently immersed in liquid nitrogen to cease the enzymatic activity. All material was stored in a freezer at -80°C for analysis of the enzymatic activity.

Extraction and incubation of the soluble invertebrates, acid invertase of the vacuole (Inv-V) and neutral invertase of the cytosol (Inv-N) were performed as described by Zeng, Wu, Avigne, and Koch (1999), and the insoluble invertases, acid invertase of the cell wall (Inv-CW), according to Cazetta, Seebauer, and Below (1999) modified by Fries (2003). Incubation was performed at zero (T0) and sixty minutes (T60). For T0, 200 μL of the sample was homogenized in Eppendorf type microtubes, and the reaction was immediately frozen on ice. For T60, 100 μL of the sample were homogenized and incubated in a water bath at 30°C for 60 minutes. Then, the reaction was frozen on ice.

The extractive and incubation of the amylases, α and β, were performed as described by Skadsen (1993), where the incubation was carried out at zero (T0) and sixty minutes (T60). For T0, 100 μL of the sample was homogenized in Eppendorf type microtubes, and the reaction was immediately frozen on ice. For T60, 100

µL of the sample was homogenized and incubated in a water bath at 30°C for 60 minutes. Then, the reaction was frozen on ice.

The enzymes were quantified by spectrophotometry at the wavelength of 540 nm, using a standard glucose curve and the 3,5-dinitrosalicylic acid (DNS) method proposed by Miller (1959). The activity of the enzymes was obtained by the difference of the values after 60 minutes of incubation of those T0. The results obtained were expressed in µmol glucose x fresh matter^{-1} x hour^{-1} (µmol gli. FM^{-1} h^{-1}).

The results were submitted to analysis of variance considering the nitrogen fertilization effect (0 and 100 kg ha^{-1}), the effect of shading (0, 30, 50, and 80% shading) as interaction sources, and nitrogen fertilization and shading. The interaction was either deployed or not deployed according to significance. The effect of nitrogen was studied by means of the F test, and the effect of shading was measured by regression analysis in which the coefficients were tested by t. $\alpha = 0.05$ (SAS, 2017) was used as the level of significance.

Results and discussion

The interaction between the different levels of shading and nitrogen fertilization was significant for the activity of the leaf, both in the 1st and 2nd cycles. In the first cycle, the highest enzymatic activity was observed when the plants were fertilized with 100 kg N ha^{-1} in 30, 50, and 80% shading. However, in full sun, or 0% shading, there were no differences between the enzymatic activity of the fertilized and nonfertilized plants. The shading had a quadratic effect in Inv-N when the plants were fertilized, presenting maximum enzymatic activity of 190.37 µmol gli. FM^{-1} h^{-1} in 53.31% shading. In the absence of fertilization, the mean activity was 61.62 µmol gli. FM^{-1} h^{-1} (Table 1).

In the second cycle, in full sun, the greatest leaflet activity was observed when the plants were fertilized. However, activity did not differ based on the level of shading. Shading had a quadratic effect whether or not the plants were fertilized. In the absence of fertilization, the maximum activity of Inv-N was 80.31 µmol gli. FM^{-1} h^{-1} in 64.67% shading, while in the fertilized plants, the maximum was 87.33 µmol gli. FM^{-1} h^{-1} at 36.43% shading.

The interaction between the different levels of shading and nitrogen fertilization was not significant for leaf Inv-V activity in either the 1st or 2nd cycles. For both cycles, the highest Inv-V activity in the leaf was observed when the plants were fertilized. In the first cycle, the shading showed a quadratic effect with maximum enzymatic activity of 164.98 µmol gli. FM^{-1} h^{-1} in 55.32% shade. However, in the second cycle, the shading did not differ, with an average enzymatic activity of 97.87 µmol gli. FM^{-1} h^{-1}.

Table 1. Activity of neutral cytosol invertase (Inv-N), acid invertase of the vacuole (Inv-V) and acidic invertase of the cell seems (Inv-CW) in Capim Marandu leaf, grown under different levels of shading (0, 30, 50, and 80%), with 0 or 100 kg of nitrogen per hectare, in two cycles.

	Fertilizing[1]	Shading				Average	CV[2]	Equations	R^2
		0	30	50	80				
					Inv-N[3]				
1st cycle	0	53.85 a	88.87 b	53.04 b	50.71 b	61.62	28.27	$\hat{Y}_0 = 61.62$	
	100	95.90 a	187.07 a	176.07 a	171.33 a	157.59		$\hat{Y}_{100} = 99.416+3.412x-0.032x^2$	0.92
	Average	74.88	137.97	114.55	111.02	109.60			
2nd cycle	0	19.71 b	59.13 a	80.80 a	76.27 a	58.98	23.52	$\hat{Y}_0 = 18.822+1.9014x-0.0147x^2$	0.98
	100	71.34 a	81.22 a	90.56 a	61.20 a	76.08		$\hat{Y}_{100} = 69.946+0.9545x-0.0131x^2$	0.87
	Average	45.53	70.18	85.68	68.73	67.53			
					Inv-V[3]				
1st cycle	0	42.67	159.26	97.44	153.25	113.16 b	34.82		
	100	108.33	206.10	161.42	161.30	159.29 a			
	Average	75.50	182.68	129.43	157.28	136.22		$\hat{Y}_x = 84.169+2.9212x-0.0264x^2$	0.60
2nd cycle	0	70.44	86.75	65.11	59.70	70.50 b	24.02		
	100	122.38	165.84	126.10	86.67	125.25 a			
	Average	96.41	126.29	95.60	73.18	97.87		$\hat{Y}_x = 97.87$	
					Inv-CW[3]				
1st cycle	0	26.11 b	11.80 b	20.39 b	55.13 a	28.36	26.84	$\hat{Y}_0 = 25.95-0.9411x+0.0163x^2$	0.99
	100	59.27 a	41.04 a	54.56 a	25.02 b	44.97		$\hat{Y}_{100} = 59.503-0.3632x$	0.64
	Average	42.69	26.42	37.47	40.08	36.67			
2nd cycle	0	9.99 b	29.36 a	29.13 a	25.18 a	23.41	41.50	$\hat{Y}_0 = 10.468+0.7966x-0.0078x^2$	0.96
	100	16.63 a	37.96 a	14.72 a	12.01 a	20.33		$\hat{Y}_{100} = 19.227+0.518x-0.008x^2$	0.46
	Average	13.31	33.66	21.92	18.60	21.87			

[1]kg N ha^{-1}; [2]Coefficient of variation; [3]µmol gli. FM^{-1} h^{-1}. Means followed by lower case letters in the column differ from each other ($p < 0.05$).

The interaction between the different levels of shading and the nitrogen fertilization was significant for the Inv-CW activity in the leaf for the two cycles. In the first cycle, the highest enzymatic activity was observed in shades 0, 30, and 50%, when the plants were fertilized. However, at 80% shading, the highest activity was observed in plants that did not receive fertilization. The shading had a quadratic influence on the enzymatic activity of plants that did not receive fertilization, with a minimum activity of 12.36 μmol gli. FM^{-1} h^{-1} at 28.87% shading. When the plants were fertilized, it presented a linear decreasing effect, gradually reducing the activity of the enzyme as the shading increased.

In the second cycle, in full sun, the highest activity of the Inv-CW in the leaf was observed when the plants were fertilized. However, the activity did not differ based on the level of shading. The shading showed a quadratic effect for Inv-CW activity in fertilized and nonfertilized plants. In the absence of fertilization, the maximum enzymatic activity, under 51.06% shading, was 30.80 μmol gli. FM^{-1} h^{-1}. However, in plants that were fertilized, the maximum enzymatic activity was 27.61 μmol gli. FM^{-1} h^{-1} in 32.37% shading.

In general, nitrogen fertilization favored greater invertase activity since the effect of the nitrogen contribution on the cellular expansion directly reflects their metabolism. Thus, there is an increase in the energy requirement for the development and growth of the plants. In chloroplasts and photosynthetic pigments, the presence of nitrogen is essential. This mineral participates in the constitution of chlorophyll *a* and *b*. In the first stage of photosynthesis, light is absorbed by a photoreceptor molecule. The main photoreceptor in chloroplasts is chlorophyll *a*, where four nitrogen atoms of the pyrroles interact with a magnesium atom (Taiz, Zeiger, Møller, & Murphy 2017). Thus, nitrogen fertilization increases the availability of this nutrient to the plant, which creates a more favorable environment for photosynthesis and photoassimilate production.

Cellular expansion of immature tissues requires hexoses as a source of both energy and carbon and depends on the direction of the cell stretching force, as a consequence of maintaining osmotic pressure and increasing the extensibility of the cell wall. Therefore, several studies have demonstrated the performance of acid invertases in the osmoregulation process (Gayler & Glasziou, 1972; Gibeaut et al., 1990). Thus, shading between 30 and 50% allowed for the maximum activity of the invertases, acting on the degradation of sucrose for use in the sites of greatest need and creating a concentration gradient between different sites discharging the carbohydrates via the phloem.

The interaction between the different levels of shading and nitrogen fertilization was significant for Inv-N enzymatic activity in the stem during the 1st and 2nd cycles. In the first cycle, in full sun and 30% shading, there was greater activity of the Inv-N in the stem of the plants that received fertilization, while those receiving 50 and 80% of shading did not show a change with fertilization. The shading showed a quadratic effect on the Inv-N in the plants that received fertilization, with a minimum activity of 97.40 μmol gli. FM^{-1} h^{-1} at 34.58% shading. In addition, in the nonfertilized plants, the shading did not differ, presenting average activity of 57.10 μmol gli. FM^{-1} h^{-1} (Table 2).

In the second cycle, with 50 and 80% shading, the highest activity of INC was observed when the plants were fertilized. The shading showed a quadratic effect for Inv-N in both fertilized and nonfertilized plants. In the absence of fertilization, the minimum activity was 32.39 μmol gli. FM^{-1} h^{-1} in 27.07% shading, and in the presence of fertilization, the minimum activity was 49.51 μmol gli. FM^{-1} h^{-1} at 26.87% shading.

The interaction between the different levels of shading and nitrogen fertilization was only significant for the enzymatic activity of the Inv-V in the stem in the first cycle. The highest Inv-V activity was observed in the plants that were fertilized at all shade levels (0, 30, 50, and 80%). In the nonfertilized plants, the degree of shading did not affect activity, presenting average activity of 76.13 μmol gli. FM^{-1} h^{-1}. However, the enzymatic activity in fertilized plants presented a linear decreasing effect, gradually reducing the activity of the enzyme as the shading increases. In the second cycle, there was no significant interaction, and fertilization and shading did not differ, with a mean activity of 67.30 μmol gli. FM^{-1} h^{-1}.

The interaction between the different levels of shading and nitrogen fertilization was not significant Inv-CW enzymatic activity in the stem, and fertilization and shading did not differ in the two cycles, displaying a mean activity of 40.01 μmol gli. FM^{-1} h^{-1} in the first cycle and 24.64 μmol gli. FM^{-1} h^{-1} in the second cycle.

Table 2. Activity of neutral cytosol invertase (Inv-N), acid invertase of the vacuole (Inv-V) and acidic invertase of the cell seems (Inv-CW) in Capim Marandu stem, cultivated under different levels of shading (0, 30, 50, and 80%), with 0 or 100 kg of nitrogen per hectare, in two cycles.

	Fertilization[1]	Shading 0	30	50	80	Average	CV[2]	Equations	R^2
					Inv-N[3]				
1st cycle	0	48.23 b	59.11 b	61.01 a	60.04 a	57.10	33.76	$\hat{Y}_0 = 57.10$	
	100	98.23 a	101.86 a	75.88 a	71.66 a	86.91		$\hat{Y}_{100} = 100.51-0.1798x+0.0026x^2$	0.75
	Average	73.23	80.48	68.44	65.85	72.00			
2nd cycle	0	41.46 a	40.48 a	32.37 b	76.68 b	47.75	23.36	$\hat{Y}_0 = 43.453-0.8173x+0.0151x^2$	0.88
	100	74.31 a	44.86 a	72.07 a	140.55 a	82.94		$\hat{Y}_{100} = 73.055-1.7521x+0.0326x^2$	0.99
	Average	57.89	42.67	52.22	108.61	65.35			
					Inv-V[3]				
1st cycle	0	60.42 b	76.09 b	78.15 b	89.86 b	76.13	44.65	$\hat{Y}_0 = 76.13$	
	100	228.69 a	182.72 a	172.21 a	161.27 a	186.22		$\hat{Y}_{100} = 219.19-0.8241x$	0.88
	Average	144.56	129.41	125.18	125.56	131.18			
2nd cycle	0	61.40	65.90	76.21	53.58	64.27 a	43.55		
	100	57.47	106.70	58.39	58.77	70.33 a			
	Average	59.44	86.30	67.30	56.17	67.30		$\hat{Y}_x = 67.30$	
					Inv-CW[3]				
1st cycle	0	46.79	39.88	39.97	22.95	37.40 a	43.11		
	100	37.09	60.56	43.23	29.60	42.62 a			
	Average	41.94	50.22	41.60	26.28	40.01		40.01	
2nd cycle	0	16.81	27.15	23.10	21.19	22.06 a	50.78		
	100	36.21	31.45	24.17	17.08	27.23 a			
	Average	26.51	29.30	23.63	19.14	24.64		$\hat{Y}_x = 24.64$	

[1]kg N ha^{-1}; [2]Coefficient of variation; [3]μmol gli. FM^{-1} h^{-1}. Means followed by lower case letters in the column differ from each other (p <0.05).

The cytoplasmic invertase is directly involved in the degradation of sucrose in the stem and in expanded tissues. In full sun and absence of fertilization, the greater activity of this enzyme is related to the osmotic regulation. This is because the plants are in direct contact with the sun, increasing the rate of transpiration and photosynthesis, as water loss is high once the stomata are open.

However, with increased shading and nitrogen fertilization, competition between intercropping plants, or even between plants of the same species may occur, as nitrogen positively influences plant growth. Thus, this high enzymatic activity supplies the energetic need of the plants via sucrose degradation, as the active photosynthetic area is reduced due to shading. Stem elongation and leaf expansion of solar radiation may also occur.

In general, the cytosol-neutral enzyme invertase (Inv-N) has a higher activity in the stem when compared to vacuole and cell wall acid invertase (Inv-V and Inv-C W). In addition, this higher neutral invertase activity is directly related to osmoregulation and stem expansion.

According to Lingle (1999), in the mature tissues of the stem, where the growth processes are almost complete, the neutral invertase becomes predominant, appearing in the cytoplasm as the concentration of the acid invertases declines. The activity of this enzyme, even with a low concentration, approximately 15 to 20% in relation to the total invertases, provides the storage of sucrose in the intercellular spaces.

The interaction between the different levels of shading and nitrogen fertilization was not significant for the activity of the Inv-N at the root, and the fertilization and shading did not differ, with a mean activity of 22.25 μmol gli. FM^{-1} h^{-1}. As was the case in Inv-CW activity in the root, presenting with a mean value of 14.26 μmol gli. FM^{-1} h^{-1}. However, the interaction between the different levels of shading and nitrogen fertilization was significant for the enzymatic activity of the Inv-V in the root. In the sol plan, the fertilization positively influenced the enzymatic activity. However, at other levels of shading, the fertilization did not cause a change enzymatic activity. The shading showed a quadratic effect on the enzymatic activity of the plants that did not receive fertilization, with a maximum value of 31.57 μmol gli. FM^{-1} h^{-1} in 60.35% shading, while in the plants that were fertilized, the minimum value was 23.11 μmol gli. FM^{-1} h^{-1} in 41.57% shading (Table 3).

Table 3. Activity of neutral cytosol invertase (Inv-N), acid invertase of the vacuole (Inv-V) and acid invertase of the cellular seems (Inv-CW) in Capim Marandu root, cultivated under different levels of shading (0, 30, 50, and 80%), with 0 or 100 kg of nitrogen per hectare.

	Fertilization[1]	Shading				Average	CV[2]	Equations	R^2
		0	30	50	80				
						INC[3]			
2nd cycle	0	17.87	23.86	24.58	23.28	22.40 a	26.4		
	100	24.40	16.32	23.93	23.75	22.10 a			
	Average	21.14	20.09	24.25	23.51	22.25		$\hat{Y}_x = 22.25$	
						IAV[3]			
2nd cycle	0	7.42 b	24.36 a	31.80 a	28.56 a	23.03	37.48	$\hat{Y}_0 = 7.1672+0.8087x-0.0067x^2$	0.99
	100	38.16 a	20.77 a	27.30 a	34.66 a	30.22		$\hat{Y}_{100} = 37.285-0.6817x+0.0082x^2$	0.86
	Average	22.79	22.56	29.55	31.61	26.63			
						IAPC[3]			
2nd cycle	0	12.71	16.00	21.64	10.77	15.28 a	51.32		
	100	20.56	13.69	13.85	12.89	15.25 a			
	Average	16.63	14.84	17.75	11.83	15.26		$\hat{Y}_x = 15.26$	

[1]kg N ha^{-1}; [2]Coefficient of variation; [3]μmol gli. FM^{-1} h^{-1}. Means followed by lower case letters in the column differ from each other ($p < 0.05$).

Due to nitrogen, which was supplied to the plant, favoring growth, there was a need for a greater mobilization of energy reserves in the root with the degradation and translocation of the sucrose to the aerial part of the plant. This was to meet the demand for stem elongation, the production of new leaves, maintaining osmotic control, avoiding dehydration and critical levels of water stress.

Sucrose is the main form of carbohydrate that is translocated in the plant via the phloem. Starch is an insoluble, reserve carbohydrate present in almost all plants. Interestingly, both sucrose and starch are generated from the triose-phosphate generated in the Calvin cycle. Invertases catalyze the highly exothermic and irreversible hydrolysis of sucrose into glucose and fructose. In contrast, a reversible cleavage of sucrose is catalyzed by sucrose synthase in UDPG and fructose, playing an important role in the metabolism of sucrose in several biochemical routes related to metabolic, structural and storage functions in plant cells (Winter & Huber, 2000).

For apoplastic discharge, the invertase enzymes are fundamental. It is necessary to maintain a low sucrose potential in the recipient cell during the unloading process so that there is no reflux of the sugar. In organs with simplelastic discharge, the low chemical potential of sucrose is maintained by respiration or by the conversion of the transported sugars into compounds required for growth or in stock polymers. In the case of organs with apoplastic discharge, the invertases play a central role in maintaining a low sucrose potential during the continuous arrival of this compound to the recipient cells (Patrick, 1997).

Reducing sugar levels in the leaf (RS_l) were influenced by shading and were associated with nitrogen fertilization, with a significant interaction in the first cycle. In full sun and 30% shading, fertilization promoted higher levels of RS_l. However, with 50 and 80% shading, the fertilization did not have an effect. The shading had an increasing linear effect on the RS_l contents of the unfertilized plants, increasing the contents gradually as the shading was increased. However, when the plants were fertilized, the linear effect decreased, reducing the contents with increasing shading (Table 4).

In the second cycle, the interaction between the different levels of shading and nitrogen fertilization was not significant for RS_l. In the shading condition, the content had a mean value of 193.28 μmol gli. FM^{-1} h^{-1}. Fertilization favored a higher reducing sugar levels in the leaves.

The reducing sugar content in the stems (RS_s) was influenced by shading and associated with nitrogen fertilization, as a significant interaction was observed in the first cycle. Fertilization promoted higher levels of RS_s in the plants at levels of 0, 30, and 50% shading. However, in 80% shading, the contents did not differ with regard to fertilization. The shading showed a quadratic effect on the contents of RS of the nonfertilized plants, with maximum values of 243.50 μmol gli. FM^{-1} h^{-1} in 48.34% shading. On the other hand, in the fertilized plants, the effect decreased linearly, reducing the contents as the shading increased. In the second cycle, the interaction between the different levels of shading and nitrogen fertilization was not significant for the RS_s. In the shading condition, the contents had an average value of 264.16 μmol gli. FM^{-1} h^{-1}. Fertilization favored higher levels of reducing sugars in the stems.

Table 4. Reducing sugar content in the leaf (RS_l), reducing sugar in the stem (RS_s) and reducing sugar in the root (RS_r) of Capim Marandu, cultivated under different levels of shading (0, 30, 50, and 80%), with 0 or 100 kg of nitrogen per hectare, in two cycles.

	Fertilization[1]	Shading				Average	CV[2]	Equations	R^2
		0	30	50	80				
Reductive sugar leaf (RS_l)[3]									
1st cycle	0	154.29b	197.45b	207.27a	240.98a	200.00	21.36	$\hat{Y}_0 = 158.05+1.0488x$	0.98
	100	263.17a	264.77a	235.80a	183.65a	236.85		$\hat{Y}_{100} = 277.68-1.0207x$	0.83
	Average	208.73	231.11	221.53	212.32	218.42			
2nd cycle	0	166.54	165.72	190.27	152.29	168.70 b	15.43		
	100	228.78	250.31	203.60	188.74	217.86 a			
	Average	197.66	208.01	196.94	170.51	193.28		$\hat{Y}_x = 193.28$	
Reductive sugar stems (RS_s)[3]									
1st cycle	0	87.13b	282.27b	184.19b	197.52a	187.78	31.60	$\hat{Y}_0 = 101.92+5.8583x-0.0606x^2$	0.62
	100	417.72a	420.82a	312.75a	214.04a	341.33		$\hat{Y}_{100} = 449.9-2.7142x$	0.86
	Average	252.43	351.54	248.47	205.78	264.55			
2nd cycle	0	251.82	230.67	232.20	236.53	237.80 b	22.32		
	100	320.27	303.82	261.51	276.45	290.51 a			
	Average	286.05	267.25	246.86	256.49	264.16		$\hat{Y}_x = 264.16$	
Reductive sugar root (RS_r)[3]									
2nd cycle	0	163.58a	210.95a	196.73a	164.78a	184.01	16.82	$\hat{Y}_0 = 165.29+2.0877x-0.0264x^2$	0.94
	100	179.72a	155.47b	153.93b	178.17a	166.82		$\hat{Y}_{100} = 166.82$	
	Average	171.65	183.21	175.33	171.48	175.42			

[1]kg N ha^{-1}; [2]Coefficient of variation; [3]μmol gli. FM^{-1} h^{-1}. Means followed by lower case letters in the column differ from each other ($p < 0.05$).

The reducing sugar content in the root (RS_r) showed a significant interaction with shade and nitrogen fertilization. The absence of fertilization favored the highest RS_r levels at 30 and 50% shading. The shading showed a quadratic effect on the RS_r contents of the nonfertilized plants, with maximum values of 208.18 μmol gli. FM^{-1} h^{-1} at 41.09% shading. However, the contents did not differ in the plants that were fertilized, with an average value of 166.82 μmol gli. FM^{-1} h^{-1}.

The reduction in the reducing sugars content in the leaves, especially in the second cycle, may be related to low photosynthetic rates. Since shading reduces the incidence of light and growth speed, resulting in a smaller leaf area, the photosynthetic rate may be compromised and photoassimilates may be produced.

The α-amylase activity in the stem was influenced by the combination of shading and nitrogen fertilization with a significant interaction observed in the second cycle. In the first cycle, the enzymatic activity did not differ with fertilization. However, shading showed a linear decreasing effect, gradually reducing the enzymatic activity as shading increased (Table 5).

In the second cycle and in full sun, the highest enzymatic activity was obtained when the plants were fertilized. The other shading levels did not differ with regard to fertilization. The shading showed a quadratic effect under the enzymatic activity of the nonfertilized plants, with a maximum value of 94.04 μmol gli. FM^{-1} h^{-1} in 42.17% shading. However, when fertilized, the effect decreased linearly.

In the roots, α-amylase activity was significantly influenced by shading combined with nitrogen fertilization. In full sun, the absence of fertilization promoted greater activity of this enzyme, while at 30 and 50% the highest activities were observed in the presence of fertilization. The shading had a quadratic effect on the enzymatic activity, both in the nonfertilized and in the fertilized plants. In the absence of fertilization, the minimum value was 0.25 μmol gli. FM^{-1} h^{-1} in 41.05% shading, and in the presence of fertilization, the maximum value was 0.76 μmol gli. FM^{-1} h^{-1} at 35.75% shading.

The activity of β-amylase in the stem was significantly influenced by shading and nitrogen fertilization in the second cycle alone. In the first cycle, nitrogen fertilization favored greater β-amylase activity in the stem. However, the shading did not have an effect, with an average value of 171.97 μmol gli. FM^{-1} h^{-1}. In the second cycle, at 80% shading, fertilization favored beta amylase activity. However, at the other shade levels, the fertilization did not have an effect. The shading produced a quadratic effect in the plants regardless of fertilization. In the absence of fertilization, the minimum value observed was 40.39 μmol gli. FM^{-1} h^{-1} at 54.34% shade, and in the presence of fertilization, the minimum value was 208.59 μmol gli. FM^{-1} h^{-1} at 24.45% shade. In the root, there was no interaction between the shading associated with nitrogen fertilization, and the fertilization did not differ at different shading levels. However, the shading showed a quadratic effect, with a minimum activity of 0.39 μmol gli. FM^{-1} h^{-1} at 40.67% shading.

Table 5. Activity of α and β-amylase on stem and root of Marandu grass, grown under different levels of shading (0, 30, 50, and 80%) with 0 or 100 kg of nitrogen per hectare in two cycles.

	Fertilization[1]	Shading				Average	CV[2]	Equations	R^2
		0	30	50	80				
α-amylase – Stem[3]									
1st cycle	0	155.86	83.98	56.93	58.21	88.75 a	70.91		
	100	120.64	257.09	89.99	72.93	135.16 a			
	Average	138.25	170.54	73.46	65.57	111.96		$\hat{Y}_x = 157.58-1.1406x$	0.57
2nd cycle	0	20.13 b	129.64 a	52.72 a	52.65 a	63.78	49.88	$\hat{Y}_0 = 30.137+3.0782x-0.0365x^2$	0.48
	100	178.90a	98.25 a	57.61 a	54.27 a	97.26		$\hat{Y}_{100} = 160.69-1.5858x$	0.85
	Average	99.51	113.94	55.16	53.46	80.52			
α- amylase – Root[3]									
2nd cycle	0	0.94 a	0.22 b	0.18 b	0.52 a	0.47	48.92	$\hat{Y}_0 = 0.934-0.0332x+0.004x^2$	0.99
	100	0.47 b	0.89 a	0.59 a	0.41 a	0.59		$\hat{Y}_{100} = 0.5048+0.0143x-0.0002x^2$	0.72
	Average	0.71	0.56	0.39	0.47	0.53			
β- amylase – Stem[3]									
1st cycle	0	101.14	65.92	90.14	123.71	95.23 b	70.07		
	100	333.31	293.99	238.74	128.82	248.72 a			
	Average	217.23	179.96	164.44	126.27	171.97		$\hat{Y}_x = 171.97$	
2nd cycle	0	322.15 a	52.52 a	84.37 a	90.09 b	137.28	60.14	$\hat{Y}_0 = 311.58-9.9789x+0.0918x^2$	0.91
	100	140.83 b	122.79 a	134.82 a	294.12 a	173.14		$\hat{Y}_{100} = 143.93-2.8903x+0.0591x^2$	0.98
	Average	231.49	87.66	109.60	192.10	155.21			
β- amylase – Root[3]									
2nd cycle	0	1.17	0.41	0.67	0.87	0.78 a	60.30		
	100	0.66	0.36	0.44	1.13	0.65 a			
	Average	0.91	0.39	0.55	1.00	0.71		$\hat{Y}_x = 0.8939-0.0244x+0.0003x^2$	0.99

[1]kg N ha^{-1}; [2]Coefficient of variation; [3]μmol gli. FM^{-1} h^{-1}. Means followed by lower case letters in the column differ from each other ($p < 0.05$).

The amount of α and β-amylase depends on several factors: variety, climatic conditions, competition for nutrients (Saika et al., 2005). In addition, with higher temperatures, the enzyme exhibited degrading activity. In this way, starch degradation is necessary when the plant is under stress. Shading and cutting (grazing) characterize a stressful factor, which impairs photosynthesis. For the plant to have an immediate recovery, it uses the degradation of its reserves, increasing the activity of these enzymes.

Thus, the leaf area remaining after defoliation is of great importance because it increases the vigor of regrowth from the immediate production of carbohydrates by photosynthesis. This results in a shorter dependence time on the reserve carbohydrates as a source of energy for the recovery of the plant.

Conclusion

Fertilization increases enzymatic activity. The invertases increase in activity when exposed to 30-50% shaded conditions, as well as in full sun, and their activity is directly linked to the osmoregulatory system. The reduction of the reducing sugars content is related to a low photosynthetic rate, and an increase in α and β-amylase is associated with the use of reserve energy sources.

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