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ORIGINAL ARTICLE

Biostimulant for sugarcane ripening in drip fertigated fields[1]

Bioestimulante para maturação de cana-de-açúcar em lavouras fertirrigadas por gotejamento

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HIGHLIGHTS:
Biostimulation with a nutritional ripener increases sugar production by sugarcane without drying off.
Nutritional ripeners anticipate the accumulation of total recoverable sugar (TRS) and sucrose.
Nutritional ripeners increase the period of industrial utilization (PIU) of sugarcane.

ABSTRACT: In irrigated fields, sugarcane's vegetative growth and carbohydrate consumption during ripening require ripener management options to improve crop yield. The hypothesis is that ripeners, together with nutrients, biostimulants, and amino acids, improve physiological responses that favor the quality of the raw material and increase the sucrose content. Consequently, the study aimed to evaluate the agro-industrial response of a fertigated sugarcane crop to ripening agents. The treatments consisted of applying chemical ripener (CR), nutritional ripener (NR), and a joint application of NR+CR, besides control with four replicates. Agro-industrial attributes (total soluble solids, apparent sucrose (AS), reducing sugars (RS), total recoverable sugar (TRS), purity, fiber, sucrose in the stalk (SS) were evaluated at 0 (before application), 12, 19, 36, and 60 days after application (DAA). The time modified the technological attributes of sugarcane under ripener application. The highest apparent sucrose and total soluble solids values were obtained with NR+CR compared to the control. The NR and NR+CR treatments presented the highest TRS contents (150 kg Mg^{-1}) at 36 DAA. Furthermore, the NR+CR treatment increased fiber content but did not compromise the quality of the raw material, and it showed high contents of SS and purity percentage, as well as low contents of RS. The chemical and nutritional ripeners efficiently promoted sugarcane ripening in areas without drying off before harvest. NR application at the end of the cycle can potentially increase sugar yield.

Key words: *Saccharum officinarum*, sugarcane industrial quality, ripeners, sugar yield, amino acids

RESUMO: Em ambientes irrigados, o crescimento vegetativo da cana-de-açúcar e o consumo de carboidratos durante a maturação requerem o manejo de maturadores para aumentar a produtividade da cultura. A combinação de maturadores, com nutrientes e bioestimulantes como os aminoácidos, pode promover respostas fisiológicas que favorecem a qualidade da matéria-prima e aumentam o teor de sacarose. Dessa forma, o estudo teve como objetivo avaliar a resposta agroindustrial da cultura de cana-de-açúcar fertirrigada aos agentes de maturação. Os tratamentos consistiram na aplicação de maturador químico (MQ), nutricional (MN), aplicação conjunta de MN+MQ e controle com quatro repetições. Os atributos agroindustriais (sólidos solúveis totais, sacarose aparente (AS), açúcares redutores (AR), açúcares totais recuperáveis (ATR), pureza, fibra, sacarose no colmo (SS) foram avaliados em 0 (antes da aplicação), 12, 19, 36 e 60 dias após aplicação (DAA). O tempo modificou os atributos tecnológicos da cana-de-açúcar sob aplicação de maturador. Os maiores valores de caldo AS e sólidos solúveis totais foram obtidos no MN+MQ em relação ao controle. Os tratamentos MN e MN+MQ apresentaram os maiores teores de ATR (150 kg Mg^{-1}), aos 36 DAA. Além disso, o tratamento MN+MQ aumentou o teor de fibra, mas não comprometeu a qualidade da matéria-prima, e apresentou altos teores de SC e pureza, bem como baixos teores de AR. Os maturadores químicos e nutricionais foram eficientes em promover o amadurecimento da cana-de-açúcar em áreas sem secagem antes da colheita. A aplicação de MN no final do ciclo tem potencial para aumentar a produtividade de açúcar.

Palavras-chave: Saccharum officinarum, qualidade industrial da cana-de-açúcar, maturadores, produção de açúcar, aminoácidos

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Introduction

Irrigation is the main management strategy adopted to minimize the negative effects of water stress on sugarcane (Dias & Sentelhas, 2019; Wonprasaid et al., 2023). The water availability to drip irrigated sugarcane during the ripening phase stimulates vegetative development, which increases carbohydrate consumption, leading to delays in maturity and a reduction in the industrial quality of the crop (Vasantha et al., 2022).

Suspension of irrigation before harvest has been used to stimulate the ripening of sugarcane (van Heerden et al., 2015; Ayele et al., 2016). However, the climatic spatio-temporal variability can compromise the success of this strategy and can cause deleterious drying to plants (van Heerden et al., 2015).

An alternative to increasing carbohydrate accumulation in sugarcane is the induction of crop maturation via chemical inducers. These chemicals induce changes in the plant's hormonal balance to inhibit vegetative growth (van Heerden et al., 2013). The trinexapac-ethyl is the main maturation inducer used in sugarcane fields in Brazil, which promotes temporary inhibition of the bioactive form of gibberellic acid (GA_1) and reduces energy consumption to growth (Van Heerden et al., 2015; Vasantha et al., 2022).

Nutrients and amino acids involved in the biosynthesis and transport of sucrose can also be used for sugarcane ripening (Jain et al., 2017; Diniz et al., 2020; Perlo et al., 2022). Previous studies showed that amino acids (as N source or hormonal precursor), phosphorus (P), potassium (K), boron (B), and magnesium (Mg) act in the synthesis and transport of sucrose and as ligands that can reduce the sucrose degradation (Pratta et al., 2011; Jain et al., 2017; Diniz et al., 2020; Perlo et al., 2022; Zolotareva et al., 2022).

This study hypothesized that the combination of ripeners with nutrients can generate a synergistic effect and enhance sugarcane maturation. Therefore, this study aimed to evaluate the response of fertigated sugarcane to the combined application of chemical ripener and nutritional ripening agents.

Material and Methods

The experiment was conducted in a commercial sugarcane area irrigated by a drip irrigation system at the Japungu Agroindustrial plant (7°6'50" S and 34°58'40" W) between April 2022 and April 2023. The plant is located in Paraiba, Brazil, and belongs to the As climate zone (Köppen classification), characterized by rainy winters and dry summers. The average annual temperature and precipitation are 26 °C and 1500 mm, respectively (Alvares et al., 2013). The accumulated precipitation during the study was 634 mm.

The study was implemented in January 2023 in plant cane cycle with the variety RB 92579 270 days after planting. The irrigation depth applied during the study was 221.2 mm, and the water supply was daily to replace the water loss by evapotranspiration. Irrigation was supplied until two weeks before harvest. This allowed the topsoil to have reduced moisture so that it would not hamper vehicle movement and the damage on the next ratoon crop apart from soil compaction (van Heerden et al., 2015). The rainfall during the study period was 390 mm (Figure 1).

The treatments included a chemical ripener (CR) [Trinexapac-ethyl at 200 g a.i. ha^{-1}], a biostimulant cited as a nutritional ripener (NR) [2 L ha^{-1}], a mixture of the chemical ripener (Trinexapac-ethyl at 200 g a.i. ha^{-1}) and nutritional ripener (2 L ha^{-1}) [NR+CR], and a control treatment, without ripener application. Product doses followed manufacturer's recommendations (Table 1).

In the sugarcane plantation selected, four treatments (ripeners) were applied in randomized stripes. Each strip was marked as CR, NR, NR+CR, and unsprayed control and contained 0.14 hectares (Pang et al., 2021). Each experimental strip comprised four replicates equidistant 20.0 m from each other, formed by seven double rows measuring 20.0 m and spaced 1.40 x 0.50 m, totaling a plot area of 266 m^2. Thus, the experiment consisted of four treatments and four replications, totaling 16 experimental units.

Ripener application was conducted 60 days before harvest (DBH). All spray mixtures were applied in the late afternoon, under appropriate humidity and wind speed conditions, by aerial equipment, an AGRAS MG type drone equipped with eight fan nozzles and a flow rate of 10 L ha^{-1}.

For the analysis of technological attributes, the stalks were sampled randomly at five different times within of experimental plot, before application (time zero) and 12, 19, 36, and 60 days after application of the treatments (DAA). During all the

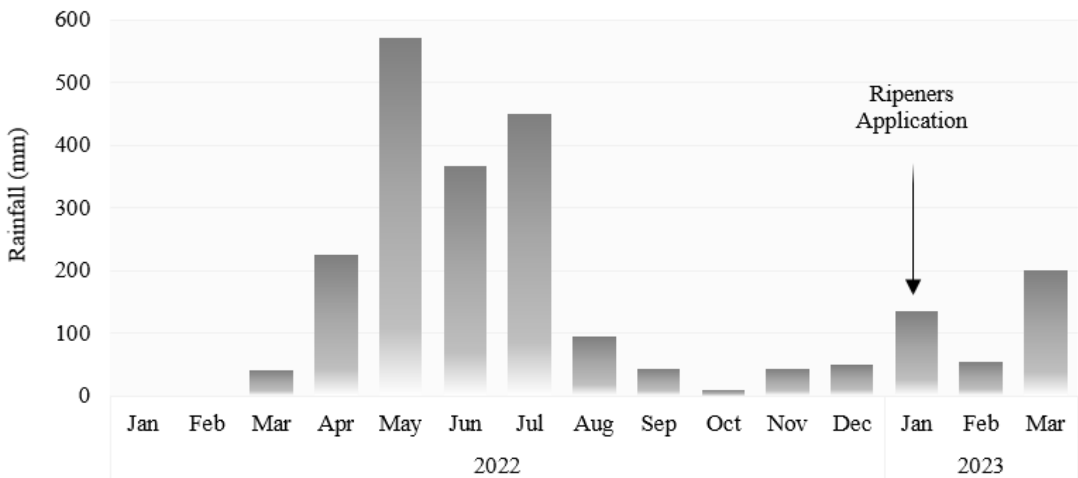

Figure 1. Rainfall during experiment conduction at Japungu Agroindustrial, PB, during the 2022/23 crop season

Table 1. Nutricional composition of the ripener

Total nitrogen (N) (g L^{-1})	23.98
Phosphorus (P_2O_5) (g L^{-1})	53.17
Potassium (K_2O) (g L^{-1})	56.36
Magnesium (MgO) (g L^{-1})	26.27
Boron (B) (g L^{-1})	8.88
Amino acids (g L^{-1})	59.0
pH	3.5
Density (g cm^3)	1.18
L-amino acid (g kg^{-1})	
Alanine	12.8
Arginine	3.5
Aspartic acid	11.9
Cystine	0.4
Glutamine	12.6
Glycine	9.0
Histidine	3.9
Hydroxyproline	0.5
Isoleucine	4.5
Leucine	5.9
Lysine	45.2
Methionine	2.6
Phenylalanine	7.7
Proline	8.2
Serine	9.5
Taurine	0.9
Threonine	9.6
Tryptophan	2.3
Tyrosine	1.6
Valine	10.6

sampling periods, sugarcane stalks were harvested manually from the sampling spots of 2 m in length for each plot.

The stalks were cut close to the ground, and the upper part was separated at the height of the apical bud, that is, at the breaking point. Then, the samples were weighed to estimate stalk yield (TSH, Mg ha^{-1}, data not shown). A subsample of 10 stalks was randomly selected and sent to the laboratory for analysis of the following characteristics: purity percentage (%), total soluble solids (TSS - °Brix) with a digital refractometer, percentage of apparent sucrose in the juice (AS) using a saccharimeter, percentage of reducing sugars (RS), fiber percentage, percentage of sucrose in the stalk (SS), and total recoverable sugar (TRS). All variables were determined based on the methodology described by CONSECANA (2006).

The normality of residuals and homoscedasticity of variances were assessed using the Shapiro-Wilk or Kolmogorov-Smirnov and Levene tests, respectively. When necessary, data were transformed to meet the assumptions of the analysis of variance. Data with normal distribution and/or homoscedasticity were submitted to ANOVA ($p \leq 0.10$).

The data relative to the number of days after treatment application were subjected to regression analysis. The regression equations selected to describe the behavior of the data were based on the best statistical adjustment using the t-test ($p \leq 0.10$).

The means results from the last evaluation time, 60 days after treatment application, were compared using the LSD test ($p \leq 0.10$). Statistical analysis was performed using SAS University Edition software.

Results and Discussion

There was a significant effect of the number of days after treatment application (DAA) on the technological attributes of sugarcane (Figure 2 and Table 2). There was a gradual increase in apparent sucrose in the juice (AS) over time in all treatments except CR (Figure 2E). The AS values were higher with the application of ripeners concerning the control in the evaluated periods. At 31 DAA, NR treatment resulted in the greatest increase in AS, with values corresponding to 18.5%.

Although there was still water availability via fertigation during the period evaluated, the AS values found in this study were high. The agronomic efficiency of the maturation process depends mainly on climatic conditions, while water supply can favor the vegetative development of sugarcane when the temperature is high (Crusciol et al., 2017). In this study, the positive results of AS indicate the additive effect of ripeners, demonstrating that they were an important tool in maturation, even in unfavorable conditions.

The maximum accumulation of total soluble solids (TSS) was found after 30 DAA (20.3° and 20.2 °Brix) with the application of CR and NR+CR, respectively (Figure 2A). Ripeners increased TSS levels by 1.6% compared to the control. The mixture of ripeners favored an increase in TSS levels; at the same time that CR based on trinexapac-ethyl reduces the level of active gibberellin, increases sucrose content, and inhibits flowering, NR has nutrients that act on protein synthesis and transport of sucrose in sugarcane (van Heerden et al., 2015; Crusciol et al., 2021).

An increase in the purity percentage of the sugarcane juice was verified with the application of ripening agents (Figure 2C). The maximum purity was obtained at 19 DAA for the treatments CR (PUR = 90.8%), NR (PUR = 91.5%), NR+CR (PUR = 90.3%), and control (PUR = 90%). These results indicated that the plant had already surpassed its maturation point during this period since mature sugarcane has juice purity values of <75% (CONSECANA, 2006).

After maturation, the purity percentage can be significantly reduced, and this behavior is observed in this study when compared with the control. The opposite response was observed with the application of ripeners, which maintained the purity levels of the juice stable up to 60 DAA (Figure 2C). Glucose and fructose are carbohydrates synthesized in the leaf and translocated through the phloem to other parts of the plant in the form of sucrose. The use of ripening agents might have induced the participation of these carbohydrates in the synthesis of sucrose, which increased the purity percentage.

The application of ripening agents contributed to the decrease in the reducing sugars (RS) values compared to the control (Figure 2B). The initial average RS values recorded were 0.4 and 0.5% for the ripeners and the control, respectively. This reduction occurred with maintenance and anticipation of the gain in sucrose when using ripeners. This is because reducing RS is detrimental to plant growth and increases the purity percentage, resulting in greater sucrose recovery efficiency (Datir et al., 2016; Vasantha et al., 2022).

The fiber content was only influenced by the application of NR+CR compared to the control (Figure 2F). The control and NR+CR gave 18 and 14% fiber contents 30 days after treatment application. The ripener induced a significant linear increase in fiber content. Leite & Crusciol (2008) also reported this increase in fiber when they evaluated the application of ripening agents on the technological quality of sugarcane.

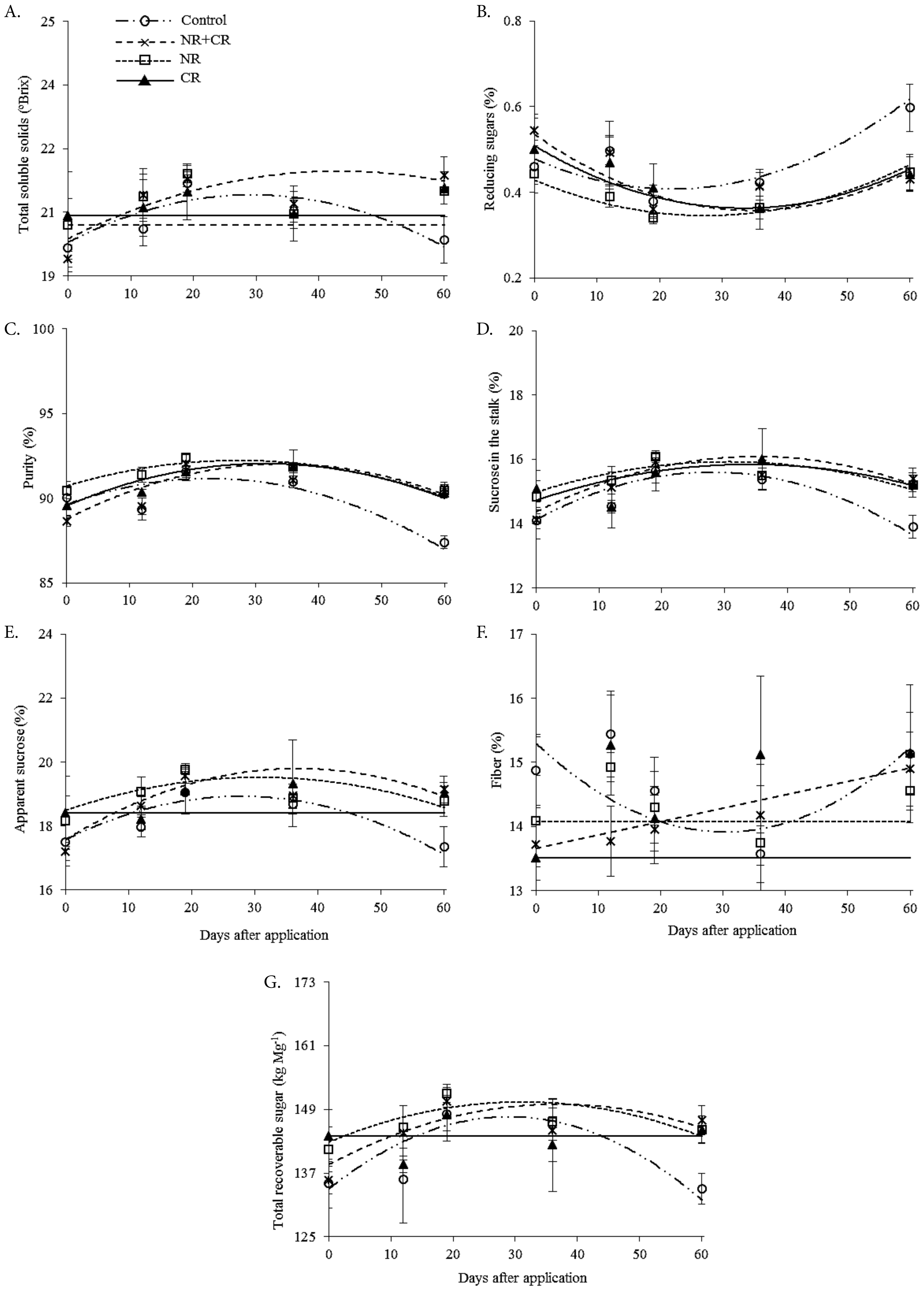

Figure 2. Total soluble solids (A), percentage of reducing sugars (B), purity percentage (C), percentage of sucrose in the stalk (D), percentage of apparent sucrose in the juice (E), fiber percentage (F), and total recoverable sugar (G) of sugarcane grown with the application of chemical and nutritional ripeners according to the number of days after application

Table 2. Equations and coefficients of determination (R^2) of the agro-industrial attributes of sugarcane with the application of chemical and nutritional ripeners

Ripeners	Equation	R^2	CV (%)
	Total soluble solids		
Control	$y = 19.789 + 0.0763^{***}x - 0.0013^{***}x^2$	0.65	1.94
Nutritional ripener (NR)	$y = 20.20^{ns}$	-	1.97
Chemical ripener (CR)	$y = 20.43^{ns}$	-	3.45
NR+CR	$y = 19.876 + 0.0736^{*}x - 0.0008^{**}x^2$	0.54	1.81
	Purity percentage		
Control	$y = 89.623 + 0.1353^{***}x + 0.003^{***}x^2$	0.83	0.85
Nutritional ripener (NR)	$y = 90.727 + 0.1091^{**}x - 0.002^{***}x^2$	0.83	0.60
Chemical ripener (CR)	$y = 89.547 + 0.1581^{**}x - 0.0025^{**}x^2$	0.96	0.99
NR+CR	$y = 88.771 + 0.1889^{***}x - 0.0028^{***}x^2$	0.68	0.95
	Percentage of apparent sucrose in the juice		
Control	$y = 17.569 + 0.0978^{***}x - 0.0018^{***}x^2$	0.85	3.20
Nutritional ripener (NR)	$y = 18.484 + 0.0675^{**}x - 0.0011^{**}x^2$	0.37	2.62
Chemical ripener (CR)	$y = 18.21^{ns}$	-	4.67
NR+CR	$y = 17.572 + 0.1208^{***}x - 0.0016^{***}x^2$	0.66	1.74
	Percentage of reducing sugars		
Control	$y = 0.478 - 0.0064^{**}x + 0.0001^{***}x^2$	0.82	9.51
Nutritional ripener (NR)	$y = 0.429 - 0.0061^{***}x + 0.0001^{***}x^2$	0.86	6.58
Chemical ripener (CR)	$y = 0.509 - 0.0087^{***}x + 0.0001^{***}x^2$	0.97	13.21
NR+CR	$y = 0.535 - 0.0102^{***}x + 0.0001^{***}x^2$	0.68	10.18
	Percentage of sucrose in the stalk		
Control	$y = 14.122 + 0.106^{***}x - 0.0019^{**}x^2$	0.89	3.14
Nutritional ripener (NR)	$y = 14.978 + 0.0618^{***}x - 0.001^{***}x^2$	0.54	2.39
Chemical ripener (CR)	$y = 14.722 + 0.0657^{**}x - 0.001^{**}x^2$	0.55	3.55
NR+CR	$y = 14.367 + 0.0981^{***}x - 0.0014^{***}x^2$	0.72	2.22
	Fiber percentage		
Control	$y = 15.297 - 0.0911^{**}x + 0.0015^{**}x^2$	0.59	4.79
Nutritional ripener (NR)	$y = 14.08^{ns}$	-	3.93
Chemical ripener (CR)	$y = 13.51^{ns}$	-	7.68
NR+CR	$y = 13.664 + 0.0207^{***}x$	0.99	4.38
	Total recoverable sugar		
Control	$y = 133.9 + 0.9407^{***}x - 0.0162^{***}x^2$	0.80	3.43
Nutritional ripener (NR)	$y = 142.8 + 0.4861^{**}x - 0.0078^{**}x^2$	0.48	2.09
Chemical ripener (CR)	$y = 144.03^{ns}$	-	3.38
NR+CR	$y = 138.6 + 0.6166^{**}x - 0.0084^{**}x^2$	0.53	2.62

Parameters with ***, **, and * have a significant effect $p < 0.01$, $p < 0.05$, and $p < 0.1$, respectively by t test

The increase in fiber content in the NR+CR treatment probably occurred due to the need to meet the plant's metabolic demand for synthesizing different compounds, such as fibrovascular bundles. The water supply might have influenced these results during the sampling periods, which favors vegetative growth and increases the fiber content of the crop. Reducing fiber content is important to increase efficiency in the industrial processing of stalks and the amount of juice extracted (Leite & Crusciol, 2008).

The treatments with NR and NR+CR outperformed the control in total recoverable sugar (TRS) accumulation until harvest (60 DAA). The treatments with ripeners reached a peak TRS of 150 kg Mg^{-1} at 36 DAA (Figure 2G). The results showed a stabilization in the trend with a subsequent reduction of TRS, which was also reported by Silva & Segato (2011). The quadratic effect in the TRS might have been due to the synchronized role of SuSy, acidic, and neutral invertases in sucrose degradation after maturation (Datir et al., 2016). However, the fast reduction in TRS for the control at 36 DAA highlights the potential of ripeners in inducing sugar translocation and storage, which increases the period of industrial utilization (PIU).

Sucrose contents were higher in the presence of plant regulators, highlighting the potential effect of these agents in promoting maturation. The ripeners increased and maintained the sucrose in the stalk (SS) at around 15%, higher than the minimum content requirement of 13% for processing (Figure 2D). Sucrose influenced the improvement of the raw material's quality, which is identified with the high levels of SS and purity and low levels of RS. The increase in sucrose content in treatments with ripeners probably occurred due to the reduction in the demand for growth organs, which ended up leading to the accelerated accumulation of sucrose in the stalk (Ayele et al., 2023b). Similar to this study, previous studies confirmed increased sucrose content due to trinexapac-ethyl treatment (Ayele et al., 2021; 2023a).

The increase in sucrose content due to the use of NR may be associated with the role of P, K, B, and Mg in the metabolism and transport of sucrose. Furthermore, it is known that metal ions such as Mg can affect sucrose accumulation by modulating the activity of sucrose metabolizing enzymes, sucrose phosphate synthase (SPS), sucrose synthase (SS), and soluble acid invertase (SAI) (Jain et al., 2013).

Jain et al. (2017) reported a rise in sucrose content and reduced SAI activity by 3 and 14%, respectively. Furthermore, SAI had also been negatively correlated with the sucrose concentration in sugarcane internodes (Datir et al., 2016). Therefore, using enzymatic effectors may have contributed to reduced SAI activity and the consequent allocation of sucrose in the stalks.

The sharp drop in sucrose content at 60 DAA (Figure 2D) for the control treatment might have been due to the availability of moisture in the soil, as irrigation was suspended just two weeks before harvest. Maintaining irrigation stimulates vegetative growth and reduces the sucrose content during ripening, resulting from the high energy demand for growth (Ayele et al., 2021).

Except for fiber content, the application of ripening agents resulted in a significant effect for all sugarcane technological variables compared to the control (Figure 3). The study also showed that the technological quality of sugarcane was affected by the use of plant regulators, and these results also agree with previous studies (van Heerden et al., 2015; Crusciol et al.; 2017; Jain et al., 2017; Ayele et al., 2021).

The age of the crop might have influenced the response to the chemical ripener. In this study, the ripeners were applied at the medium maturation stage (270 DAP). Ayele et al. (2023b) reported higher sucrose contents and yields in sugarcane harvested at 240 and 300 DAP, while lower responsiveness to ripeners was recorded in later applications. According to the

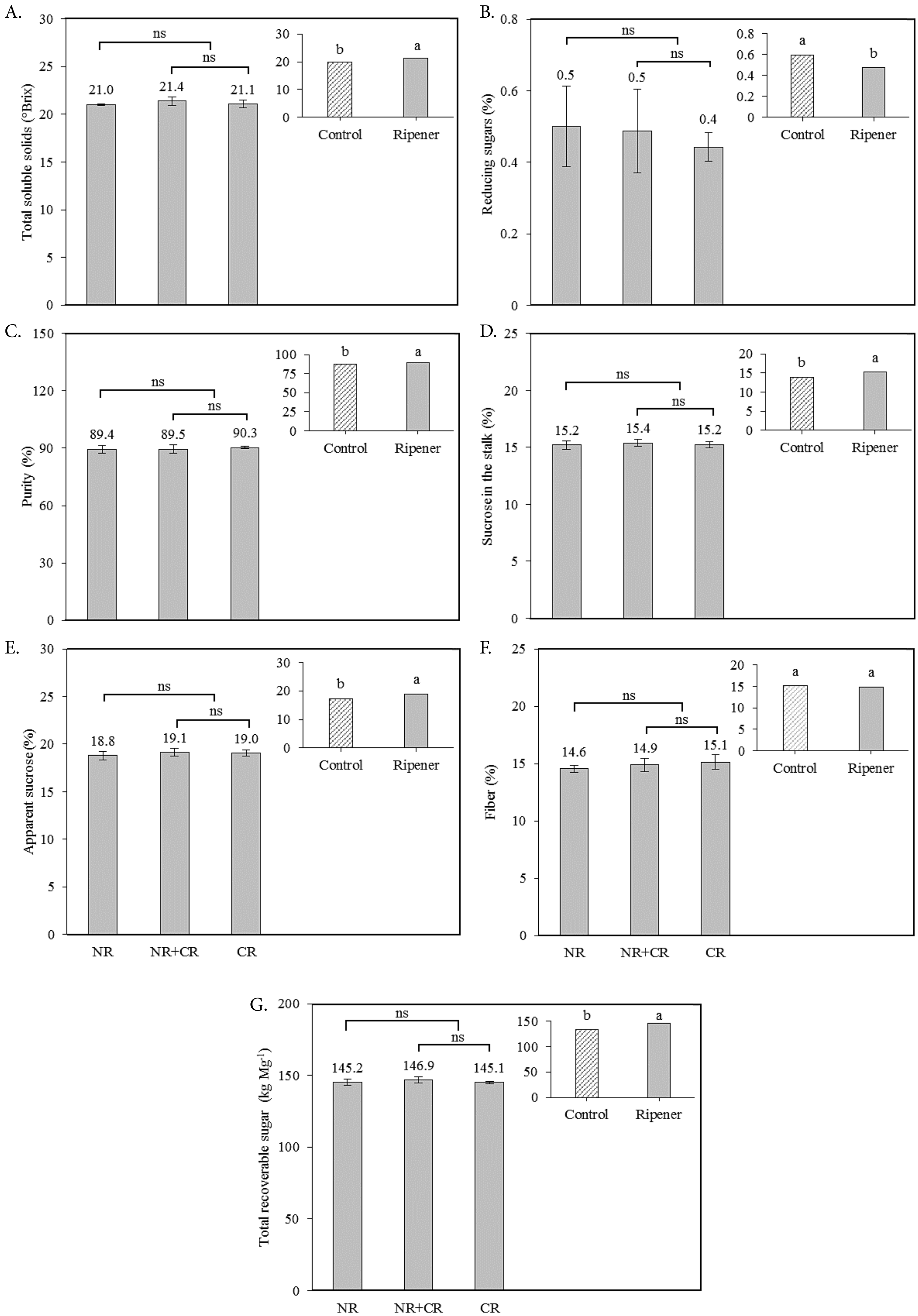

Figure 3. Total soluble solids (A), percentage of reducing sugars (B), purity percentage (C), percentage of sucrose in the stalk (D), percentage of apparent sucrose in the juice (E), fiber percentage (F), and total recoverable sugar (G) after 60 days of application of chemical and nutritional ripeners. Different letters between treatments indicate a significant effect $p \leq 0.10$ by the LSD test. Nutritional ripener (NR), Chemical ripener (CR), Nutritional ripener + Chemical ripener (NR+CR)

authors, this decrease could be due to the increase in relative maturity as age increases from 300 DAP. This is important, as ripeners favor a more uniform accumulation of sucrose in the internodes of the apical region, which are generally immature, which is an absolute prerequisite to obtaining the maximum benefits from chemical ripening.

Maintaining irrigation up to two weeks before harvest may have contributed to the greater positive effect of ripeners compared to the control. It is known that the reduction of water availability in the soil is an important inducer of natural maturation; however, the dry-off and ripener effects on sugarcane quality are not additive. Thus, the maintenance of soil moisture through irrigation associated with the use of ripening agents has increased the accumulation of sucrose in the stalks since compounds such as trinexapac-ethyl need to be absorbed by the leaves and converted into their active form to inhibit the precursor of sucrose (GA_1) and consequently increases the allocation of sucrose in immature stalks (van Heerden et al., 2013).

It was found that there is no difference between the ripeners for the analyzed variables, indicating that both are efficient in promoting maturation (Figure 3). Thus, these agents favored the maturation of sugarcane and contributed to the accumulation of sugar in reserve tissues.

Conclusion

1. The ripeners were capable of increasing the total soluble solids, total recoverable sugar, purity, sucrose in the stalk, and apparent sucrose compared to the control. The quality of the raw material increased due to the greater sucrose accumulation in reserve tissues.

2. Sugarcane maturation with combined application of NR+CR was similar to treatments with isolated application of NR and CR. Therefore, both application methods efficiently promote crop maturation without imposing drying.

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