ADRIANA CONCEIÇÃO DA SILVA

ASPECTOS FISIOLÓGICOS E AGROINDUSTRIAIS DE CLONES DE CANA-DE-AÇÚCAR SOB CULTIVO DE SEQUEIRO

TERESINA – PI

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Tese apresentada à Universidade Federal do Piauí, como parte das exigências do Programa de Pós-Graduação em Agronomia - Agricultura Tropical, para obtenção do título de Doutor em Ciências.

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A Deus que sempre esteve comigo, dando-me forças nesta jornada.

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RESUMO GERAL

O déficit hídrico é um dos componentes ambientais que mais limita a produção da cana-de-açúcar, principalmente em áreas não irrigadas onde a restrição hídrica ocorre em períodos de pleno crescimento e desenvolvimento da cultura. O acúmulo de osmorreguladores em resposta a essa limitação torna-se um mecanismo importante para manter o turgor celular, mitigando a redução do potencial hídrico. Este estudo teve como objetivo avaliar clones de cana-de-açúcar sob cultivo de sequeiro no principal polo de cultivo de cana-de-açúcar do Estado do Piauí, com base em parâmetros biométricos, bioquímico-fisiológicos, tecnológicos e de produtividade. Dividido em capítulos, o estudo abordou o referencial teórico no capítulo I e, no capítulo II, tratou de investigar a eficiência fotossintética e regulação osmolítica na tolerância de seis clones de cana-de-açúcar (RB975375, RB006629, RB955977, RB011549, RB064109 e RB064125) e duas variedades comerciais (RB867515 e RB92579) cultivados em condições semiáridas. Os clones RB975375, RB006629, RB064109, e RB064125, sob cultivo com limitação hídrica, exibiram melhor crescimento e resposta às trocas gasosas e osmóticas. Os clones RB975375 e RB064109 apresentam alta eficiência no uso da água, refletindo melhor rendimento de colmo e açúcar comparado às variedades RB867515 e RB92579. O capítulo III versou sobre os osmoprotetores e rendimento tecnológico em clones de cana-de-açúcar cultivados em ambientes com limitação de água, que teve como objetivo avaliar a variação temporal da concentração de carboidratos e a produtividade dos clones de cana cultivados em ambiente de sequeiro, em dois ciclos de cultivo (2017/18 e 2018/19). Foram avaliados os clones RB975375, RB021754, RB955977, RB064125, RB006995, RB068027 e duas variedades comerciais (RB867515 e RB92579). Os clones RB021754, RB064125, RB068027, RB006995 e RB975375 apresentam expressivo acúmulo de osmoprotetores sob restrição hídrica, no entanto os clones RB068027 e RB975375 apresentaram alta eficiência de uso da água nos ciclos cana-planta e cana-soca, além de maior rendimento de colmo e açúcar.

Palavras Chave: Carboidratos, déficit hídrico, eficiência do uso da água, prolina, *Saccharum spp*.

ABSTRACT

Water deficit is one of the environmental components that most limits sugarcane production, especially in non-irrigated areas where water restriction occurs during periods of full growth and crop development. The accumulation of osmoregulators in response to this limitation becomes an important mechanism to maintain cell turgor, mitigating the reduction in water potential. This study aimed to evaluate sugarcane clones under rainfed cultivation in the main sugarcane cultivation pole in the Brazilian state of Piauí, based on biometric, biochemical, physiological, technological and productivity parameters. Divided into chapters, the study addressed the theoretical framework in Chapter I, and in Chapter II it investigated the photosynthetic efficiency and osmolytic regulation in the tolerance of six sugarcane clones (RB975375, RB006629, RB955977, RB011549, RB064109 and RB064125) and two commercial varieties (RB867515 and RB92579) grown in semi-arid conditions. Clones RB975375, RB006629, RB064109, and RB064125, under cultivation with water limitation, exhibited better growth and response to gas and osmotic exchanges. Clones RB975375 and RB064109 showed high efficiency in the use of water, reflecting better stalk and sugar yields compared to varieties RB867515 and RB92579. Chapter III evaluated osmoprotectors and technological performance in sugarcane clones grown in water-limited environments, which aimed to evaluate the temporal variation of carbohydrate concentrations and the productivity of sugarcane clones grown in rainfed environments.

Keywords: Carbohydrates, water deficit, water use efficiency, proline, Saccharum spp.

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LISTA DE ABREVIATURAS

A - Taxa de Assimilação
A/CI - Eficiência Instantânea de carboxilação
A/E - Eficiência Instantânea de uso de água
A/GS - Eficiência Intrínseco de uso de água
ATR - Açúcar Total Recuperável
CI - Concentração Interna de Carbono
COMVAP - Usina Comvap Açúcar e Álcool
DAC – Dias Após o Corte
DAP - Dias Após o Plantio
DAP - Dias após o plantio
E - Transpiração
ET0 - Evapotranspiração
EUA - Eficiência no Uso da Água
GS - Condutância Estomática
Kc - Coeficiente de Cultivo
MCW - Metanol, Clorofórmio e Água
PCA - Análise de Componentes Principais
PCC - Pol%
PSII - Fotossistema II
PZA - Pureza Aparente do Suco
RIDESA - Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético
STAR - Amido
SUC - Sacarose
SYH - Rendimento de Colmos por Hectare
TRS – Açúcar Total Recuperável
TSC – Carboidratos Soluveis
WUE - Eficiência do Uso de Água

Sumário

1. INTRODUÇÃO GERAL

A cana-de-açúcar (*Saccharum* spp.) é uma cultura de grande importância econômica para o Brasil, onde os colmos são fonte de matéria-prima para a produção de açúcar, álcool e outros subprodutos oriundos do processamento da indústria sucroenergética. Cultivada em diversos estados brasileiros (MAIA JUNIOR et al., 2020), o Estado do Piauí, na safra de 2018/2019, produziu 839,5 mil toneladas e produtividade agrícola estimada em 57,5 Mg ha^{-1}, (CONAB, 2019). Em particular, esse baixo desempenho se deu em função da irregularidade das chuvas e a acentuados veranicos ocorridos durante o ciclo de cultivo.

O regime pluviométrico de uma região é o principal elemento influenciador dos cultivos agrícolas, de maneira que a ocorrência de déficit hídrico impacta negativamente a produção agrícola em todo o mundo. A cana-de-açúcar é sensivelmente influenciada pela baixa disponibilidade hídrica do solo, comprometendo o rendimento final quando esse tipo de estresse ocorre em determinadas fases ou durante todo o ciclo de desenvolvimento (SINGH, 2020). Em meio a essa realidade, o setor canavieiro do Piauí tem buscado desenvolver e aproveitar as áreas de sequeiro através de genótipos de cana-de-açúcar que sejam capazes de suportar, com maior estabilidade, o déficit hídrico comum à região. Entretanto, os estudos de campo acerca da variação no resultado ao regime de sequeiro ainda são incipientes.

Quando submetidas ao déficit hídrico os clones da cana-de-açúcar induzem alterações no potencial hídrico das folhas, em consequência, alterações fisiológicas e bioquímicas serão responsáveis por reduzir a condutância estomática e a taxa fotossintética, aumentando a concentração de substâncias osmoprotetores (prolina, trealose e glicina betaína), visando manter o turgor celular (WILLADINO et al., 2010).

Além disso, o metabolismo das proteínas fica comprometido levando a mudanças na proporção de aminoácidos e aumentos significativos na concentração de prolina. Compreende-se que a quantidade de osmoprotetores pode diferir entre clones ao longo do ciclo de cultivo. Contudo, não está claro se os picos de produção de osmoprotetores ocorrem durante o ciclo da cultura e se, essa informação, pode ser útil para embasar a escolha dos clones de cana-de-açúcar melhor adaptados ao cultivo em sequeiro.

Alguns estudos, visando investigar os mecanismos bioquímicos e fisiológicos de clones de cana-de-açúcar cultivadas em regime de sequeiro, foram realizados por

Carlin et al., 2009; Maia Júnior et al., 2020; Srivastava; Kumar et al., 2020 e Garcia et al.,2020. No estanto, por terem sido conduzidos sob condições controladas, os resultados não retratam a realidade do campo, onde os vários elementos climáticos encontram-se presentes e influenciam diretamente o comportamento dos clones.

Diante do exposto, o presente estudo objetivou avaliar clones de cana-de-açúcar cultivados em regime de sequeiro no principal polo canavieiro do Estado do Piauí, com base em parâmetros biométricos, bioquímico-fisiológicos, tecnológicos e produtivos.

CAPÍTULO I

2. REFERENCIAL TEÓRICO

2.1- Importância da cultura da cana-de-açúcar no estado do Piauí

O Brasil destaca-se como maior produtor e exportador mundial de cana-de-açúcar em virtude do desenvolvimento de um agronegócio forte, por meio do aumento da demanda mundial dos produtos que a cana-de-açúcar é matéria-prima. Aliado às grandes áreas cultiváveis e às condições edafoclimáticas favoráveis ao cultivo, o país é participante competitivo no mercado mundial (CONAB, 2019).

A região Nordeste é caracterizada como promissora ao desenvolvimento do setor Sucroenergético do país, apresentando expansão das áreas de cultivo da cultura. Nessa região, as características climáticas favorecem o cultivo da cana-de-açúcar, em virturde da sua exigência de luz e água (ANDRADE JUNIOR et al., 2018). Apesar do cultivo consolidado nos Estados de Alagoas, Pernambuco e Paraíba, a expansão da cana-de-açúcar em regiões não tradicionais, como o Piauí, é decorrente das características de solo, topografia, clima e logística de exportações favoráveis (TREVISAN et al., 2019).

Neste cenário, o Estado do Piauí destaca-se com áreas de expressivo potencial produtivo, alcançando 22.600 hectares na safra 2018/19 (CONAB, 2020). Apresentando clima semiárido e subúmido seco, com precipitações que variam de 800 a 1.200 mm por ano, a irregularidade de chuvas durante parte ou em todo ciclo da cultura é o principal gargalo para obtenção de altas produtividades nessa região (SILVA et al., 2019). Em virtude dessas condições hídricas como fator limitante à cultura, a identificação de clones de cana-de-açúcar que tolerem serem cultivados em ambientes de sequeiro é necessária (ANDRADE JUNIOR et al., 2017).

2.2- Regime de sequeiro

Grande parte das atividades humanas sofrem profundas influências das condições climáticas, em especial as atividades agrícolas que são vulneráveis às irregularidades pluviométricas, principalmente em áreas onde se predomina a agricultura de sequeiro (DIN et al., 2019).

Considera-se que o regime de sequeiro é um modelo de cultivo dependente exclusivamente da precipitação local para o crescimento e desenvolvimento das plantas, onde a implantação das culturas agrícolas ocorre durante o início do período chuvoso. Muitas vezes, o regime pluviométrico da região não é suficiente para atender a demanda hídrica da cultura, seja em parte ou durante o ciclo completo da cultura presente no campo (QIAO et al., 2018). Nesse sentido, cultivos de sequeiro são considerados de alto risco, visto a fragilidade desses sistemas de produção, que se refletem nas oscilações das áreas colhidas, produções, produtividade, além dos preços e renda associada a essas atividades (BEDEKE et al., 2019).

Em meio aos principais cultivos sob regime de sequeiro, a cana-de-açucar é praticada no semiárido nordestino do Brasil por grande parte dos agricultores (LEMOS et al., 2020). Estudo realizado por Sánchez-Román et al. (2015), avaliando o desempenho produtivo da cana-de-açúcar submetida diferentes reposições hídricas (100, 75, 50, 25 e 0%), com e sem aplicação de nitrogênio na água utilizada na irrigação por gotejamento superficial em cana planta e soca, observou-se incrementos de 15,68 e 4,32% na produtividade de colmos e ATR para a cana planta e de 31,28 e 1,04% para a cana soca.

Andrade Junior et al. (2017), avaliando a produtividade de variedades de cana-de-açucar sob diferentes formas de manejo de irrigação, observaram que as variedades de cana-de-açúcar RB962962 e RB867515 apresentaram 162,3 e 158,5 Mg ha^{-1} de rendimento de colmos, respectivamente, em todos os sistemas de manejo, inclusive no sistema de gotejamento subsuperficial. Estes estudos demonstram que apesar de vários fatores poderem influenciar o rendimento de colmo, a reposição hídrica no solo é essencial para a evolução das taxas de produtividade da cultura da cana-de-açúcar (CORREA et al., 2019).

2.3 - Crescimento e produtividade de cana-de-açucar sob restrição hídrica

O desafio atual do cultivo de cana-de-açúcar para alcançar maiores produtividades consiste em superar os efeitos deletérios da limitação hídrica. Os períodos de sua deficiência são cada vez mais extensos e, ainda mais prejudiciais ao cultivo, quando são realizados em solos com baixa capacidade de retenção hídrica (LEAKEY et al., 2019).

As fases de crescimento mais afetadas pelo déficit hídrico na cana-de-açúcar são o perfilhamento e desenvolvimento dos colmos. As áreas destinadas à produção de alimentos dependem de fatores locais, como clima e solo; embora o déficit hídrico sozinho, ou combinado com outros estresses abióticos restrinja mais as culturas agrícolas que qualquer fator ambiental isolado. Este fato faz com que a tolerância à seca das culturas destinadas à produção de alimentos, torne-se um fator primordial para a exploração de áreas agrícolas (KAUR et al., 2019).

Conforme descrito por Pincelli et al. (2012) que, avaliando respostas de variáveis morfológicas de quatro cultivares comerciais de cana-de-açúcar (SP81-3250, SP83-2847, RB855453 e RB72454) sob dois regimes hídricos, observaram que essas cultivares se comportavam de forma diferenciada à deficiência hídrica em relação as suas características morfológicas foliares. Nesse contexto, verificaram que os clones SP813250 e SP832847 quando submetidos à deficiência hídrica por período prolongado no início do desenvolvimento, apresentavam maior largura de folhas, menor dano ao número de folhas verdes e área foliar, aumento na densidade estomática nas superfícies foliares e maior produção de massa de matéria seca, as quais foram consideradas tolerantes para essa condição.

De acordo com Endres et al. (2018), a altura da planta, o comprimento da folha, o número de folhas verdes e a área foliar são indicadores morfológicos potencialmente confiáveis para selecionar clones de cana-de-açúcar tolerantes ao déficit hídrico. Nesse estudo, os dados obtidos revelaram que o rendimento de colmo está correlacionado com a altura de plantas, comprimento e diâmetro dos entrenós. Esses resultados são corroborados por aqueles encontrados por Mirajkar et al., 2019, onde a análise de trilha revelou maior efeito positivo direto da altura da planta sobre o rendimento de colmo.

Hemaprabha et al. (2013), visando identificar genótipos tolerantes à seca, estudar o padrão de segregação para a seca de híbridos elite de cana-de-açúcar e progênies de um cruzamento comercial comprovado e, analisando os atributos de produção de açúcar e parâmetros bioquímicos em condições normais e de seca observaram redução na altura das plantas (35,05%) e comprimento dos entrenós (25,13%) nas progênies.

O aumento da incidência e intensidade do déficit hídrico severo levou os programas de melhoramento da cultura da cana-de-açucar a investirem em diversas ações para gerar novos clones eficientes no uso da água e tolerantes ao estresse hídrico. O plantio de cultivares de cana-de-açúcar tolerantes ao déficit hídrico caracteriza-se como ferramenta preventiva para reduzir os danos causados por esse tipo de estresse. Diversos estudos têm sido conduzidos com esse propósito e focando as alterações fisiológicas imediatas que são causadas pela restrição hídrica, como o fechamento estomático parcial e redução do potencial hídrico (GONG et al., 2020).

A limitação hídrica afeta consideravelmente a eficiência fotossintética nas folhas da cana-de-açúcar que, na maioria das vezes, são acompanhados de baixa área e alongamento de folhas e caules, principalmente pela redução gradual da expansão celular, que é imposta pelo déficit hídrico (ANJUM et al., 2017).

As altas temperaturas são geralmente favoráveis à atividade fotossintética, entretanto quando combinadas com déficit hídrico pode aumentar a velocidade de transpiração que, muitas vezes se torna maior que a captação de água pelas raízes, pode promover respostas diferentes entre as cultivares (IQBAL et al., 2020).

Fisiologicamente, quando as plantas são submetidas ao déficit hídrico, a taxa fotossintética é uma das variáveis que apresenta maior sensibilidade à limitação de água no solo (SOUSA et al., 2020). Porém, as plantas podem continuar realizando fotossíntese por tempo superior àquele destinado ao crescimento em expansão, pois os estômatos respondem mais lentamente no início do estresse hídrico que o turgor celular (TARDIEU et al., 2012). Dessa forma, ocorre redução na condutância estomática à assimilação de CO_2, consecutivamente à atividade fotoquímica que irá causar alterações nas taxas de crescimento, refletindo consideravelmente na produção.

Com concentrações intercelulares de CO_2 mais baixas, a fotossíntese é fortemente limitada. Nesse caso, a planta precisa equilibrar a necessidade de conservar água e assimilar CO_2 atmosférico, fazendo que a área foliar desempenhe papel importante na difusão do dióxido de carbono e no vapor de água entre os estômatos. Transpiração e fotossíntese, com a consequente acumulação de massa seca estão relacionadas às trocas gasosas entre os estômatos e a atmosfera (HARRISON et al., 2020).

A tolerância ao déficit hídrico em algumas variedades de cana-de-açúcar está, em geral, associada à manutenção da integridade da membrana celular, sendo essas protegidas contra lesões causadas por condições de déficit hídrico (SÁNCHEZ-ELORDI et al., 2020).

2.4- Deficiência hídrica do solo e o metabolismo do carbono

Os cultivos agrícolas estão sujeitos à períodos de deficiência hídrica no solo, de maneira que a redução na turgescência celular é suficiente para o fechamento dos estômatos, limitando a absorção de dióxido de carbono e diminuindo sensivelmente a atividade de assimilação de carbono, paralisando o crescimento das plantas e reduzindo a biossíntese proteica (LIMA-MELO et al., 2019; OSHUNSANYA et al., 2019).

A taxa de assimilação líquida de carbono é determinada pelas características bioquímicas, fisiológicas e morfológicas da maquinaria fotossintética, variando com as condições do ambiente tais como: irradiância; temperatura, concentração de dióxido de carbono (CO_2), suprimento de água e nutrientes (MOHOTTI; LAWLOR, 2012).

Por meio do controle estomático, as plantas diminuem a perda de água e reduz a assimilação fotossintética, sendo necessário o uso das reservas acumuladas (SIMÕES et al., 2019). O acúmulo de amido nas folhas ocorre quando há paralisação na exportação de triose-fosfato do cloroplasto e, normalmente, está associado à redução da força do dreno e dos teores de sacarose no tecido (PAUL et al., 2003; AINSWORTH; BUSH et al., 2011). Neste sentido, o amido de reserva é hidrolisado pela atividade das amilases, havendo maior acúmulo de carboidratos solúveis, aminoácidos e ácidos orgânicos que serão utilizados na respiração, crescimento e síntese de novos tecidos (VOLENEC et al., 2020). O amido transitório é sintetizado nos cloroplastos das plantas superiores como resultado do excesso de carbono recém-assimilado (SMITH; ZEEMAN et al., 2020).

A assimilação da planta, em geral, é suficiente para suprir, não somente a demanda imediata do crescimento (a partir da síntese e mobilização de sacarose), mas para acumular compostos na folha (síntese de amido transitório) (SMITH; ZEEMAN et al., 2020). Durante a noite subsequente ao período de assimilação, o amido transitório é degradado, gerando substratos que serão utilizados na síntese de sacarose. Esse açúcar fornece esqueletos de carbono e energia tanto para as células foliares como para os tecidos não fotossintetizantes (MACNEILL et al., 2017). Dessa forma, a degradação do amido durante o período noturno auxilia na provisão de carbono necessário ao crescimento da planta. Esse argumento foi corroborado por Sales et al. (2015) que avaliando plantas de cana-de-açúcar sob condições de estresse radicular, demonstrou que o teor foliar de carboidratos não estruturais, sacarose e amido nas plantas é aumentando sob frio de raiz.

2.5 - Mecanismos de tolerância ao déficit hídrico

As plantas desenvolveram várias estratégias de tolerância à seca como: mudanças no ciclo de vida, modulação do crescimento e desenvolvimento (para combinar com o suprimento limitado de água), regulação de funções da planta, ajustes na alocação de recursos para o crescimento durante o estresse ou a evolução da percepção do sinal de estresse, e a longo prazo, a tolerância ao estresse (HIRAYAMA et al., 2010; HU et al., 2014; YOU et al., 2015).

A base de conhecimento em expansão ajudou a identificar os principais genes associados à tolerância à seca e à manutenção do crescimento. Toda essa expansão possibilita o desenvolvimento de condições de déficit hídrico em várias culturas, incluindo a cana-de-açúcar (AUGUSTINE et al., 2015; RAMIRO et al., 2016). A cana é uma cultura tropical com metabolismo fotossintético C4 em que, sob estresse hídrico moderado, ocorre diminuição na condutância estomática (*gs*), na taxa de transpiração (*E*), na concentração interna de CO_2 (*Ci)* e na taxa fotossintética. Isso sucede principalmente pelas limitações estomáticas, que juntamente com a inibição do crescimento de colmo e folhas, pode ser considerada adaptação inicial mais comum quando os clones de cana-de-açúcar são submetidas à desidratação leve à moderada (BASNAYAKE et al., 2015).

A suscetibilidade da cana-de-açúcar à limitação de água no solo é maior nas fases de perfilhamento e alongamento do caule, visto que o crescimento do colmo e folhas são mais afetados. Esse tipo de estresse, quando moderado na fase de maturação, tem efeitos positivos sobre o rendimento de açúcar (sacarose) devido a fotossíntese ser menos sensível ao estresse que o crescimento das plantas, canalizando o CO_2 assimilado para a produção de sacarose e acumulação de sólidos solúveis no colmo (MACHADO et al., 2009; LAKSHMANAN; ROBINSON et al., 2014).

Além disso, sob deficiência hídrica, a divisão e o alongamento celular são interrompidos (MACHADO et al., 2009; INMAN-BAMBER, 2005). O desenvolvimento radicular também é influenciado pelo déficit hídrico, mas relativamente menor que a biomassa acima do solo (INMAN-BAMBER et al., 2008). O declínio induzido pelo estresse hídrico na taxa fotossintética é causado principalmente por reduções na atividade da fosfoenolpiruvato carboxilase (PEPcase) e, posteriormente, na atividade da ribulose-1,5-bifosfato carboxilase (Rubisco) (LAKSHMANAN; ROBINSON, 2014). Vale ressaltar que a taxa de fotossíntese também é impactada pelo acúmulo de açúcar nas folhas, pois, sob condições não estressantes, o baixo teor de açúcar nas folhas é propício à fotossíntese, enquanto o alto teor de açúcar modera a fixação de carbono (FERREIRA et al., 2017).

A produção e o transporte de fotoassimilados nas plantas são regulados pela atividade fotossintética e pela força do dreno. A fotossíntese está associada à maior demanda por carbono pelos colmos, por se tratar de um dreno de alta prioridade na alocação dos fotoassimilados que, mesmo em períodos curtos de restrição hídrica, pode comprometer o acúmulo de sacarose no colmo (HENNION et al., 2019).

Assim, a redução no teor foliar de amido foliar em plantas sob déficit hídrico sugere que as plantas utilizam-se das reservas foliares disponíveis para suprir a demanda do dreno e/ou arcar com os custos metabólicos da manutenção da homeostase sob condição estressante. Níveis aumentados de açúcares como a trealose podem estar envolvidos nas respostas ao déficit hídrico que reduzem os danos à membrana celular. Assim, a capacidade de acumular trealose foi observada em raízes de cana-de-açúcar sob limitação hídrica, ocasionando aumento na hidrólise de amido e propiciando níveis mais elevados de açúcares solúveis (FÀBREGAS et al., 2019).

Na avaliação de Santos et al. (2019), a tolerância à seca de variedades de cana-de-açúcar em diferentes fenofases, possibilita identificar características fisiológicas e bioquímicas associadas à menor perda de produtividade em condições de déficit hídrico. Observaram ainda que as variedades RB867515 e RB92579 não sofreram alterações significativas no potencial osmótico ao meio-dia, durante o estresse hídrico na fase de crescimento intenso, apesar do baixo potencial hídrico foliar e da pouca variação nos teores de osmorreguladores. Por esses considerandos, essas duas variedades apresentaram maiores potenciais de adaptação à ambientes com pouco fornecimento de água, apresentando também maior rendimento produtivo quando submetidas às condições de seca.

Ainda na pesquisa de Santos et al. (2019), observou-se que as variedades mais afetadas pela limitação hidrica das diferentes fenofases foram RB855536 e RB855113, ocorrendo maiores reduções no potencial hídrico e osmótico, e maior acúmulo de osmorregulador em resposta à seca, mas não o suficiente para evitar a desidratação, o que provavelmente contribuiu para o menor desempenho produtivo.

Para sobreviver e crescer sob estresse, as plantas desenvolvem diferentes mecanismos adaptativos: escape, retardo e tolerância à desidratação.

2.5.1- Escape

Em geral, plantas que se desenvolvem em climas com sazonalidade acentuada são capazes de adaptar-se às condições ambientais flutuantes, aumentando sua eficiência para essas condições limitantes (BARON; BÉLANGER et al., 2020).

Uma das estratégias consiste em escapar do estresse hídrico, por um curto ciclo de colheita, permitindo que as plantas concluam seu ciclo antes que o estresse hídrico severo se instale (BYKOVA et al., 2019).

2.5.2- Retardo

O retardo à desidratação envolve mecanismos voltados para manutenção do nível de hidratação celular pela baixa condutância estomática durante a condição de estresse (BLUM, 2005; KOOYERS, 2015).

Os mecanismos de prevenção são características importantes em áreas com déficit hídrico, pois aumentam as chances de capturar ao máximo a umidade do solo, limitando a perda de água e retendo a hidratação celular, permitindo, portanto, a recuperação da colheita quando o estresse é aliviado.

De acordo com o estudo desenvolvido por Inman-Bamber et al. (2012), avaliando variedades de cana-de-açúcar para ambientes com limitação hídrica, observaram o aumento da profundidade do enraizamento e da eficiência intrínseca do uso da água e, em menor extensão, ocorreu redução da condutância, levando ao aumento da eficiência de transpiração. Tais efeitos são sugeridos como as melhores características a serem consideradas para a seleção de clones de cana-de-açúcar em ambientes com limitação de água nos trópicos e subtrópicos. No entanto, sob estresse hídrico, esses mecanismos reduzem o acúmulo de biomassa por meio de grande redução na área foliar e fixação de carbono (BLUM 2005; TARDIEU, 2012; COMINELLI et al., 2013).

2.5.3-Tolerância

A tolerância à seca é um mecanismo que permite às plantas manter o metabolismo, tolerando a redução do potencial hídrico dos tecidos sob déficit hídrico, principalmente pelo acúmulo de solutos compatíveis ou osmólitos, proteínas osmoprotetoras e pela capacidade antioxidante (SAXENA et al., 2019).

Mecanismos de tolerância são características favoráveis, em condições de déficit hídrico leve e moderado, pois permitem a manutenção do crescimento durante o estresse. As características de tolerância estão diretamente ligadas à alta condutância estomática, sustentando a taxa de fotossíntese e também a tolerância ao estresse térmico ao diminuir a temperatura das folhas (BLUM, 2005; TARDIEU, 2012; COMINELLI et al., 2013).

Oliveira et al. (2010) avaliando o crescimento e a produtividade de onze (11) variedades de cana-de-açúcar submetidas à irrigação total, verificaram maiores taxas de perfilhamento na variedade RB92579. Zhao et al. (2013), também observaram que essa variedade foi mais tolerante ao ambiente de sequeiro, apresentando maiores taxas de perfilhamento sob estresse hídrico. Esses autores afirmaram ainda que o perfilhamento e o comprimento do colmo podem ser indicadores úteis para detectar precocemente clones de cana-de-açúcar tolerantes ao estresse hídrico.

Como discutido acima, os mecanismos de tolerância à desidratação ajudam a alcançar melhor crescimento e rendimento de colheita, embora o(s) mecanismo(s) exato(s) de tolerância ao estresse hídrico não sejam totalmente compreendidos na cana-de-açúcar (SILVA et al., 2012; BASNAYAKE et al., 2015).

Algumas dessas características estão envolvidas no melhor desempenho das culturas sob estresse leve a moderado, entre as quais podemos citar: maior número perfilhos, altura e peso de colmo, o diâmetro do colmo (dependente do genótipo que do ambiente), teor de clorofila foliar (índice SPAD), temperatura das folhas e dossel, taxa de fotossíntese, condutância estomática e taxa de transpiração (SILVA et al., 2012; BASNAYAKE et al., 2015). Sendo que essas características ainda podem ser utilizados como critério de seleção indireta para genótipos de cana-de-açúcar tolerantes ao estresse hídrico, entre outros (SILVA et al., 2012; BASNAYAKE et al., 2015).

2.5.4- Eficiência do uso da água

A eficiência no uso da água (kg m^{-3}) é definida como a relação entre a produtividade da cultura (kg ha^{-1}) e o volume total de água consumido nos processos fisiológicos de produção (m^3 ha^{-1}). Observa-se que o consumo anual de água pela cana-de-açúcar pode variar de 1.500 a 2.500 mm (15.000 a 25.000 m^3/ha) (LEAL et al., 2017).

A disponibilidade hídrica adequada pode reduzir, significativamente, as perdas e proporcionar ainda condições favoráveis ao desenvolvimento vegetativo da planta. Por ser uma planta C4, a cultura da cana-de-açúcar possui alta eficiência fotossintética com elevada saturação de luz (LEAKEY et al., 2019). Desde modo, quando a demanda hídrica está adequada, os incrementos na produtividade podem ser atribuídos ao fornecimento de água durante todo o período de crescimento, proporcionando maior acesso aos nutrientes, via sistema radicular da cultura.

Como resultado, ocorre a remobilização de energia e de carboidratos das raízes, para o aumento do perfilhamento e o alongamento do colmo (INMAN-BAMBER et al., 2005), que é estimulado pela produção de hormônios vegetais, responsáveis pelo crescimento vegetativo da parte aérea das plantas (TAKEI et al., 2002).

Plantas que possuem melhor controle da função estomática são mais tolerantes à seca, já que os estômatos podem ser regulados com base no nível de déficit hídrico, fechando apenas parcialmente os estômatos. Nesse sentido, levando a ocorrer alguma fixação de carbono durante as condições de seca e ao aumento na eficiência do uso da água (SANTOS et al., 2019).

Estudos anteriores mostram que a eficiência das trocas gasosas, nas plantas que possuem maior capacidade fotossintética, resulta em maior eficiência do uso da água e em maior eficiência intrínseca, correlacionada positivamente com a manutenção da produtividade, durante os períodos de seca, em diversos cultivos agrícolas, incluindo a cana-de-açúcar (LEAKEY et al., 2019).

2.6 - Acúmulo de osmoprotetores e a limitação hídrica

A capacidade das plantas se recuperarem do estresse hídrico, após a reidratação, está fortemente ligada aos mecanismos de tolerância acionados durante o estresse (ZHANG et al., 2019). A produção de osmoprotetores, como prolina, glicina betaína, entre outros, em plantas estressadas, ajuda a mitigar os danos fotoquímicos do PSII, permitindo melhor recuperação pós-seca.

Em conformidade com a pesquisa realizado por Santos et al. (2015), que avaliando alterações fisiológicas associadas às enzimas antioxidantes em resposta à tolerância da cana-de-açúcar ao déficit de água e reidratação, concluíram que o déficit hídrico aumentou os níveis de prolina e das enzimas superóxido dismutase e ascorbato peroxidase em todas as cultivares. Nesse estudo, observou-se ainda, o aumento de carboidratos sob estresse, exceto na cultivar SP81-3250, que apresentou teor reduzido. Devido aos menores danos causados pela seca na fisiologia e na bioquímica das cultivares SP83-2847 e IAC91-5155, elas apresentam maior potencial de tolerância à ambientes com limitação hídrica.

Em geral, nas regiões tropicais, o estresse hídrico é associado ao aumento da radiação solar e temperatura, o que faz aumentar ainda mais a energia que chega aos fotossistema, causando fotoinibição e fotossíntese reduzida (ZHANG et al., 2019; GUIDI et al., 2019).

Quando ocorre a fotoinibição, a energia luminosa excede a quantidade de energia utilizada na fotossíntese, causando declínio na eficiência quântica efetiva do PSII (VENDAS et al., 2013; TRUJILLO et al., 2013; GUIDI et al., 2019).

O acúmulo de osmorreguladores, dependendo de suas concentrações, é indicado como um mecanismo auxiliar que contribui, para manter a turgidez celular, mesmo com baixo potencial hídrico (ZHANG et al., 2019). Embora alguns estudos já tenham investigado o acúmulo de osmorreguladores, na tolerância à seca da cana-de-açúcar, pouco se sabe sobre sua ação em conjunto com regulação da atividade do PSII em resposta ao estresse e pós-estresse na cana-de-açúcar (SANTOS et al., 2015; ABBAS et al., 2014; VANTINI et al., 2016).

A prolina está ligada a mecanismos que previnem a perda de água nas plantas, por meio de ajuste osmótico, atuando como osmorregulador celular. Esses benefícios podem ser decorrentes da compartimentação subcelular entre citosol, cloroplastos e mitocôndrias, permitindo que funções específicas sejam executadas nesses diferentes compartimentos celulares (KAUR et al., 2015).

Conforme Vantini et al. (2016), que avaliou os acúmulos em níveis de prolina nas raízes de duas cultivares de cana-de-açúcar submetidas à seca, uma tolerante (RB867515) e outra sensível (SP86-155), o maior acúmulo de prolina na cultivar tolerante comprovou seu caráter de tolerância à seca. Esse osmoprotetor ajudou as plantas a manterem sua turgidez após cinco dias de déficit hídrico (estresse moderado) e a lidar com o déficit hídrico por 10 dias (estresse severo) quando comparadas com a planta sensível. O resultado deste trabalho indica que o metabolismo da prolina acelerou os processos de sinalização celular que promoveram a turgidez celular, favorecendo a sobrevivência da cultivar tolerante por um maior período de déficit hídrico.

Em trabalho conduzido por Abbas et al. (2014), avaliando 13 genótipos de cana-de-açúcar expostos a diferentes intensidades de limitação hídrica, seguido por um período de reidratação, verificou-se que as concentrações de prolina, glicina betaína, peroxidação lipídica e conteúdos fenólicos na folha, frente a diferentes doses de polietilenoglicol, aumentaram quando a concentração de PEG aumentou. Esses resultados reafirmam os estudos realizados com cana-de-açúcar, onde altas concentrações de prolina atuaram como antioxidantes, diminuindo o estresse oxidativo e contribuindo para o equilíbrio fotoquímico dos cloroplastos (HEMAPRABHA et al., 2013; SANTOS et al., 2015).

O acúmulo de açúcar solúvel também pode estar associado a mecanismos que evitam a perda de água nas plantas como ajuste osmótico. Estudos mostram que o total de carboidratos solúveis permaneceu constante nas folhas e aumentou nas raízes de plantas de cana-de-açúcar submetidas à seca (HAYAT et al., 2010; SALES et al., 2015).

Segundo esses autores, os teores de carboidratos solúveis permanecem constantes, provavelmente pela degradação das reservas de amido. Os níveis aumentados de carboidratos solúveis totais também foram observados na cana sob estresse hídrico e salino (PATADE et al., 2011).

Solutos como carboidratos solúveis, prolina, glicina betaína, entre outros, podem ser acumulados em altos níveis, em muitos casos, sob baixos potenciais hídricos, no entanto, sem causar nenhum transtorno à função proteica, visto que são hidrofílicos e podem proteger a célula contra a desidratação (FORLANI et al., 2019).

A osmorregulação, por si só, não é responsável pelo crescimento, pois a turgescência gerada não constitui o único fator de controle, além da elasticidade da parede celular e, por conseguinte, do coeficiente de sua elasticidade (ROJAS et al., 2017). O ajustamento osmótico visa promover o turgor necessário ao crescimento, mas não ocorrendo o ajustamento da elasticidade da parede celular, isto é, aumentando a sua capacidade de extensão, não haverá crescimento (SÁNCHEZ-BLANCO et al., 2019).

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CAPÍTULO II

Photosynthetic efficiency and osmolytic regulation in the tolerance of sugarcane clones grown in semi-arid conditions

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Abstract

High performance of sugarcane under drought establishes a sound basis to select promisor genitors to cultivate in soils with water restrictions. The hypothesis that drought tolerance and productivity in sugarcane clones are closely related to control of photosynthetic efficiency and osmotic adjustment in different phenophases of the crop was tested in this study. The experiments were carried out under rainfed regime in a commercial area of Sugar and Alcohol Industry Ltda. The treatments were arranged in completely randomized blocks, consisting of six promising (RB975375, RB006629, RB955977, RB011549, RB064109, and RB064125) and two commercial (RB867515 and RB92579) sugarcane clones. Overall, the experimental area displayed low rainfall associated with increases in the air temperature, especially from 60 to 150 days after planting (DAP), resulting in a noticeable increase of evapotranspiration. In addition to superior photosynthetic performance, the RB006629 promising sugarcane clone displayed higher values of instantaneous and intrinsic water-use and carboxylation efficiencies, emerging as the sugarcane clone with best physiological performance among all clones studied. The PCA performed with biometric and biochemical parameters obtained at 150 DAP (during drought) explains that 65% of the total data variation and shows the inverse correlation between starch and free proline. The promising sugarcane clones, mainly RB975375, RB006629, RB064109, and RB064125, exhibited better growth, gas exchange and osmotic responses under conditions of water limitations. Among them, the RB975375 and RB064109 display better technological traits and higher stalk and sugar yields, emerging as the most potential clones to cultivate in semiarid regions.

Keywords: Carbohydrates, Drought, Osmoprotectants, Photosynthesis, *Saccharum* spp

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1. Introduction

Sugarcane (*Saccharum* spp.) is a grass species widely explored in several countries, playing a crucial role in the world economy by providing raw material for sugar and alcohol production (Sanghera et al., 2019). The agricultural areas in tropical regions have been drastically affected by climatic changes, demanding rapid solutions of breeding programs to develop genotypes capable of grown under abiotic stresses (Raza et al., 2019). The water deficit has been cited as the biggest limiting trait to plant growth and development, mainly when associated with high temperatures (Zhao and Li 2015; Joshi et al., 2016; Begcy and Dresselhaus 2018).

Studies have been conducted to develop or investigate drought-tolerant sugarcane varieties in semiarid regions (Gentile et al., 2015; Endres et al., 2018). The deleterious effects of the water limitation are more pronounced in the early stages of sugarcane cultivation (up to 200 days after planting), represented by the stages of sprouting, tillering, and vegetative growth (Kumar et al., 2019). According to Vasantha et al. (2020), sugarcane clones show a 56% reduction in production when grown under water limitation (50% of the volume and frequency of full irrigation).

Under drought stress, sugarcane usually shows elevated control of stomatal conductance and favorable carbon assimilation, displaying better water use efficiency and growth (Barbosa et al., 2015; Zhao and Li 2015). In this limiting environment, stomatal closure constitutes the first defense mechanism to prevent excessive loss of water by transpiration (Araújo et al., 2019). Consequently, stomatal regulation can culminate in the increased mobilization of reserve carbohydrates to act as a substrate for metabolism and synthesis of compatible osmolytes (Robert et al., 2015). Water deficit also impairs the hydraulic conductivity of leaves, implying in low leaf water potential and amplifying its effect along with the entire plant (Barbosa et al., 2015; Santos et al., 2019).

Osmoregulation emerged as an essential trait against drought harmful effects (Abbas et al., 2014; Anjum et al., 2017). Proline is a crucial osmoregulator that contributes to osmotic adjustment in many stressful situations, especially in drought (Hayat et al., 2012). This molecule protects the cell membranes and metabolites and can be used as a source of carbon and nitrogen after the plant reaches the homeostasis (Hemaprabha et al., 2013; Vantini et al. 2016). Additionally, other compatible solutes, like soluble sugars, glycine-betaine, and free amino acids, have been cited as key compounds during water stress responses (Abbas et al., 2014; Santos et al., 2015). The solute accumulation decreases the osmotic and water potential of cells protecting the plants against dehydration and constituting a vital acclimation mechanism for drought tolerance (Basu et al., 2016; Farooq et al., 2017).

Our hypothesis is that drought tolerance and productivity of sugarcane clones are closely related to better photosynthetic efficiency and osmotic adjustment in different phenophases of this crop. To assess this hypothesis, we investigated the performance of promising and commercial clones of sugarcane in a semiarid region with water restrictions. Physiological, biochemical, and technological parameters were studied in order to identify and select drought-tolerant sugarcane clones.

2. Materials and Methods

2.1 Characterization of the experimental area

The experiments were carried out under dryland conditions, during 2017/2018 season, in an area of commercial sugarcane production from Sugar and Alcohol Industry Ltda (COMVAP) in the municipality of União, Piauí State, Brazil (4°52′09″S and 42°52′45W), at a mean altitude of 112 m. The climate in the region is sub-humid dry, mega-thermal, with moderate water stress in the summer (Bastos and Andrade Júnior 2014). During the experimental period, from 30 to 300 DAP, environmental conditions (air temperature and rainfall) were registered through an automatic agrometeorological station and the data were employed to estimate the reference evapotranspiration (ET_0) according to Penman-Monteith's method.

Soil humidity was weekly measured (on Monday, Wednesday and Thursday) from the topsoil layer up to 60 cm depth using a capacitance probe (Diviner 2000®, Sentek Pty Ltd., Adelaide, SA). Two PVC tubes installed separately throughout each experimental block, totaling six across the experimental area, were used to insert the probe and determine weekly readings. Ground samples were collected to measure the volumetric humidity (θ) and correlate with scaled frequency (SF) readings for calibration purposes (Silva et al., 2007). The values of SF were converted to water percentage in the ground (θ) through the equation $\theta v = 0.796\ (SF)^{3.69}$ ($R^2 = 0.89$). The soil chemical and physical characterization was performed in composite samples formed from the collection of simple samples randomly collected and at three depths 0.0 to 0.2, 0.2 to 0.4, and 0.4 to 0.6 m, respectively (Table 1).

2.2 Growth conditions and treatments

Sugarcane Genetic Breeding Program of RIDESA (Inter-University Network for the Development of Sugarcane Industry; www.ridesa.com.br) developed the promising clones used in this study. The field experiment was carried out in a randomized complete block design with eight treatments, being six promising (RB975375, RB006629, RB955977, RB011549, RB064109, and RB064125) and two commercial clones widely cultivated in the Northeast region (RB867515 and RB92579) – with three replicates.

Each experimental plot consisted of four 10.0-meter rows, spaced 1.4 m, totaling 56.0 m². Only two central rows were used for assessments, disregarding 0.5 m at the extremities to avoid the border effect, and the useful area of each plot was 25.2 m². The plantation was performed manually, distributing six sugarcane propagules with three buds for meter, totaling 18 buds per meter of each sugarcane plant per furrow. The base fertilization was implemented after soil chemical analysis and 500 kg ha^{-1} of NPK (06-28-22) was applied in the planting furrow.

2.3 Gas exchange, plant growth, and biochemical analyses

Gas exchange was measured using a portable infrared gas analyzer (LI-6400XT, LiCor® Biosciences, Lincoln, Nebraska, USA) at 90 DAP (period with the highest soil water restriction). The CO_2 assimilation rate (A; μmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mol H_2O m^{-2} s^{-1}) and internal CO_2 concentration (Ci; μmol mol^{-1}) were assayed between 7:30 and 9:00 am, and readings were taken from the mid region of the fully expanded +3 leaf. Based on these data, instantaneous water-use efficiency (A/E; μmol CO_2 mmol^{-1} H_2O), intrinsic water-use efficiency (A/gs; μmol CO_2 mol H_2O) and instantaneous carboxylation efficiency (A/Ci; μmol CO_2 m^{-2} s^{-1} Pa^{-1}) were calculated.

The plant growth was determined at 90 and 150 DAP in three aleatory plants per plot. The following plant growth parameters were recorded: stem diameter, plant height, and leaf area index. The leaf area index was measured through an LAI-2000 Plant Canopy Analyzer (LiCor® Biosciences, Lincoln, Nebraska, USA) by taking four readings (one above the canopy and three at ground level) from each treatment. At 60 and 150 DAP, samples of sugarcane leaves were collected, stored at -20 ºC, and, posteriorly, used to quantify the concentration of total soluble carbohydrates (Dubois et al., 1956), starch (Dubois et al., 1956; Amaral et al., 2007), and free proline (Bates et al., 1973).

2.4 Agro-industrial and yield of sugarcane

Sugarcane yield and agro-industrial attributes were determined at 420 DAP. The useful area of each plot was harvested, and the sugarcane plants were manually cut, without burning, and stalks were separated. Ten stems of sugarcane were randomly collected in the user area to measure the agro-industrial attributes. The following technological quality parameters were analyzed in the Laboratory of COMVAP (CONSECANA 2007): apparent purity (%), sucrose and fiber content (%), and total recoverable sugar (Mg ha^{-1}). The stalks biomass was measured with a dynamometer, and the stalk yield (Mg ha^{-1}) was quantified. The sugar yield (Mg ha^{-1}) was calculated by multiplying stalk yield with sucrose content and dividing by 100, according to proposed by Lima Neto et al., (2013).

2.5 Statistical analysis

The data were subjected to analysis of variance (ANOVA) and, when necessary, the mains were compared by Scott Knott's test ($p > 0.05$), using the statistical program SISVAR® 5.3 (Ferreira, 2011). The principal component analysis was performed with normalized data using Statistica software (Statsoft, 2004).

3. Results

3.1 Climatic conditions during the experiments

In this study, the air temperature during all experiments varied between 25.8 and 29.1 °C (Fig. 1), constituting an ideal condition for sugarcane growth and development (Silva et al., 2019). The accumulated rainfall during the full sugarcane cycle was 1,890.8 mm, being observed the highest volume of rainfall in April 2017 (407.6 mm) and February 2018 (318.0 mm) (Fig. 1). Nonetheless, only 25.8 mm were registered between 90 (June 10, 2017) and 150 (September 10, 2017) DAP. The reduced rainfall was associated with increases in the air temperature, especially from 60 to 150 DAP, which resulted in a noticeable increase of evapotranspiration (ET0).

The soil humidity varied during the crop cycle, being reduced in all soil layers between 40 to 200 DAP (Fig. 2). The soil moisture at 0.0 to 0.1 m decreased and achieved values lower than critical humidity in sprouting (phenophase I). This soil layer displayed lower soil humidity in tillering (phenophase II) when compared to other soil layers. On the contrary, the soil layer between 0.1 and 0.4 m displayed a highest water level in tillering. During vegetative growth (phenophase III), the soil moisture was inferior to critical humidity until 220 DAP (29.1% of soil moisture), i.e., there was 135 days of water deficit in this period. In maturation (phenophase IV), the water level in 0.0 to 0.4 m soil layer was increased to values superior to critical humidity after rainfall in 220 DAP. In contrast, the soil layer between 0.4 to 0.6 m was maintained inferior to critical humidity. Overall, the soil humidity was sharply increased in 0.0 to 0.6 m soil layer in maturation (Fig. 2).

3.2 Gas exchange responses of sugarcane clones under drought

Gas exchange parameters from sugarcane clones were measured at 90 DAP, and data are shown in Fig. 5. The RB006629 promising sugarcane clone displayed the highest values of CO_2 assimilation rate, transpiration rate, and stomatal conductance (Fig. 3). In contrast, the RB867515 clone exhibited lower photosynthetic performance besides decreases stomatal conductance and transpiration values (Fig. 3A-3C). The highest and lowest intracellular CO_2 concentrations were observed in RB064125 and RB92579 clones, respectively (Fig. 3D). In addition to superior photosynthesis, the RB006629 clone displays higher values of instantaneous and intrinsic water-use and carboxylation efficiencies (Fig. 4), figuring as the sugarcane clone with best physiological performance among all clones studied.

The RB867515 commercial clone displayed high intrinsic water-use efficiency (Fig. 4B); however, this clone showed lower values of photosynthesis, transpiration, stomatal conductance, and instantaneous carboxylation efficiency; thereby it was considered the most sensitive clone to water limitation.

The principal component analysis was performed using gas exchange parameters measured at 90 DAP and explain 73.6% of the total variation (Fig. 5). Overall, there was possible to identify the similarity between sugarcane clones using the PCA, being noticeable in the formation of two distinct groups. The RB006629, RB064125, RB064109, and RB92579 clones were clustered into group I, while RB975375, RB011549, RB955977, and RB867515 clones were assembled into group II. The sugarcane clones clustered into group I show a positive relationship mainly with photosynthesis, transpiration, stomatal conductance, and intracellular CO_2 concentrations (Fig. 5). On the other hand, the second group displays a lower correlation with gas exchange parameters analyzed, except with intrinsic water-use efficiency, and this response evidences higher sensitivity of these clones to drought.

3.3 Plant growth and biochemical analyses

In general, the RB006629, RB955977, and RB064125 sugarcane clones displayed plant height superior to other plants in 90 and 150 DAP (Fig. 6A). In absolute values, the RB975375 was the most affected by water limitation and displayed the lowest plant height (2.02 m) at 150 DAP. While the stem diameter was not significantly affected by water restriction (Fig. 6B), the leaf area index was significantly affected by water disponibility (Fig. 6C). At 90 DAP, the RB006629, RB955977, RB011549, and RB064125 promising clones exhibited higher values of leaf area index and were superior to commercial clones (RB867515 and RB92579). The RB006629, RB955977, and RB064109 promising clones display higher values leaf area index at 150 DAP, being 144% superior to commercial clones RB867515 and RB92579 in the same period. The RB011549 and the commercial clones (RB867515 and RB92579) were most affected by water restriction at 150 DAP and displayed the lowest values of the leaf area index (Fig. 6C).

Considerable variation in total soluble carbohydrates, starch and free proline contents in response to water limitation was registered in sugarcane clones (Fig. 7). Sugarcane clones displays lower values of total soluble carbohydrates and starch content at 60 DAP (before the water deprivation) than the values recorded at 150 DAP (in drought) (Fig. 8A). The RB064125 clone displayed higher total soluble carbohydrates at 60 DAP, while RB955977 showed the lowest value of this parameter in the same period (Fig. 7A). The higher and lower values of starch content at 60 DAP were recorded in RB064109 and RB867515 clones, respectively. At 150 DAP, the RB867515 commercial clone exhibited higher starch content compared to others sugarcane clones (Fig. 7B).

Free proline accumulation was differentially regulated in sugarcane clones, except for RB011549 that showed similar free proline levels in the two evaluated periods (Fig. 7C). In general, free proline content was increased by water deficit, i.e., more free proline was observed at 150 DAP when compared to 60 DAP, except for

RB006629, RB955977 and RB064125 sugarcane clones that displays a reduction in this amino acid in response to water stress (Fig. 7C). The highest concentration of free proline was recorded in the RB975375 clone at 60 and 150 DAP. The PCA performed with biometric and biochemical parameters obtained at 150 DAP (during drought) explains that 65% of the total data variation and showed an inverse correlation between starch and free proline (Fig. 8). It is possible to note that the RB867515 commercial clone was separated from the other clones and displays a lower correlation with all parameters analyzed, mainly leaf area index, total soluble carbohydrates, and free proline. These results indicate that this clone was more sensitive to water stress conditions than other clones evaluated in this study.

3.4 Agro-industrial attributes and yield of sugarcane clones

Agro-industrial attributes and yield of sugarcane clones are shown in table 2. There was no difference between the sugarcane clones for the apparent purity and sucrose content, but fiber content varied significantly. The highest fiber content was registered in RB064109 (16%), while the lowest value was observed in RB975375 (12%). The RB975375 promising clone stood out in terms of total recoverable sugar (152.5 Mg ha^{-1}) and overcame the two commercial clones (RB867515 and RB92579). Unexpectedly, the RB064109 clone exhibited the lowest value of total recoverable sugar (120.8 Mg ha^{-1}). The stalk and sugar yield was similar between RB006629 promising clone and RB867515 and RB92579 commercial clones and lower than others clones (Table 2). The RB975375 promising clone exhibited higher sugar yield (17.1 Mg ha^{-1}) in comparison to other tested sugarcane clones.

4. Discussion

4.1 Promising sugarcane clones for water limiting environments

The performance of sugarcane is powerfully in environments with a better moisture distribution throughout the production cycle, with water demand between 1,500 and 2,500 mm per year (Teixeira et al., 2016). According to Ferreira Júnior et al. (2015), the biomass accumulation and stalk yield have a linear relationship with solar energy absorbed and water consumed by the crop. Herein, during the experimental period, the field conditions showed an excess of rainfall in April 2017, with subsequent decrease in humidity and an evapotranspiration that remained above the rainfall volume during seven months (Fig. 1). Thus, the data indicate that precipitation lower than evapotranspiration along the year generated a stressful environment for sugarcane clones. Nevertheless, sugarcane clones exhibited improved resilience to long and severe exposure to drought at certain development stages.

The environmental conditions had significant effects on most of the biometric characteristics analyzed, especially in RB955977, RB064125, RB064109, and RB006629 clones, indicating a excellent adaptation to water restriction (Figs. 6A,B and C). The plant growth parameters revealed that sugarcane yield could be positively associated with most agronomic variables (Fig. 6; Table 2). Khan et al. (2015) observed that increase in sugarcane yield under water limitations was associated with better biometric characteristics, such as plant height, length of internodes and stem diameter, being the plant height direct and positively correlated with sugarcane yield. In concordance with, sugarcane clones with greater plant height and leaf area index exhibited better sugar yield (Figs.6A and C). These results reinforce the importance of biometric characteristics as indicators of water stress tolerance in sugarcane, as proposed by Silva et al. (2012). Commercial sugarcane clones (RB867515 and RB92579) exhibited poor development and stalk yield under low water availability (Fig. 6; Table 2).

Growth of sugarcane plants was previously found to be reduced by up to 80% under water deficit conditions (Endres et al., 2018). Physiological events and parameters related to cellular turgidities, such as cell elongation, leaf area, and root growth, are the first to express negative responses to water restriction, and therefore are considered the first defense mechanisms underlying the drought (Souri et al., 2020). Also, the reduced cell elongation in response to water restriction provokes decrease in leaf expansion, stem development, and therefore plant growth (Bakht et al., 2020). Concordantly, the decrease in growth of RB867515 and RB92579 commercial clones (Fig. 6) negatively influenced in the sugar yield and quality (Table 2).

4.2 Photosynthetic performance of sugarcane clones under water limitation

Herein, physiological evaluations were performed in sugarcane clones at 90 DAP, corresponding the most unfavorable time-period to plant growth, evidenced by low soil humidity and high evapotranspiration (Fig. 1 and 2). Overall, RB006629, RB064109 and RB064125 clones exhibited elevated performance, mainly higher CO_2 assimilation and transpiration rates, stomatal conductance, stem diameter, and leaf area index (Fig. 3 and 6). Also, transpiration rate and stomatal conductance displayed positive correlation (Pearson' coefficient (r) = 0.96), suggesting a crucial mechanism for control the hydric status under water deficiency.

In general, the molecular, biochemical, and physiological mechanisms involved in sugarcane responses to water stress are complex, and information more accurate of these mechanisms is fundamental for a better understanding of drought resistance strategies (Guidi et al., 2019; Santos et al., 2019). In this study, the clones RB006629 and RB064125 displayed different performance and maintained the stomatal openning even under low water availability. On the other hand, some sugarcane clones showed the lowest values of transpiration and stomatal conductance, mainly RB867515 commercial clone, which resulted in elevated sensitivity to water deficiency.

Surprisingly, the RB006629 promising clone displayed higher photosynthesis, transpiration and stomatal conductance combined with greater higher water-use and carboxylation efficiencies (Fig. 3 and 4). On the other hand, the RB867515 commercial clone displayed the opposite performance. This phenomenon seems to be attributed to imbalance between photochemical and biochemical phases of photosynthesis as result of the reduction in Calvin-Benson cycle activity (Khorobrykh et al., 2020). Thus, our results suggest that RB006629 promising clone displayed the best photosynthetic efficiency under drought and therefore is most adequate to environment with water limitations.

4.3 Free proline is a key piece for drought tolerance of sugarcane clones

Several studies have suggested multifaceted roles for proline, including compatible osmolyte of cell turgor (Anjum et al., 2017) and antioxidant compound, capable to reduce the oxidative stress and contribute to the photochemical balance of chloroplasts (Hemaprabha et al., 2013; Santos and Silva 2015). The physiological role of free proline in sugarcane grown under drought was evaluated by Rhein et al. (2011), Abbas et al. (2014), Santos and Silva (2015) and Vantini et al. (2016). For all cases, the proline accumulation was cited to help with osmotic adjustment in plants subjected to drought.

Here, RB975375 promising clone exhibited higher proline under drought in comparison to commercial clones (RB867515 and RB92579). Thus, the higher levels of free proline in RB975375 clone most likely help to maintain the chloroplast structural integrity and minimize injuries in photosystems caused by water deprivation. As a result, photosynthetic rates of these clones were maintained higher even with lower water availability when compared to other clones. The promising sugarcane clones, mainly RB006629, RB975375, RB064109, and RB064125 clones, displayed greater CO_2 assimilation, and stalk and sugar yield when compared to commercial sugarcane clones (RB867515 and RB92579).

4.4 Organic solutes and sugarcane productivity under water deficit

Sugarcane plants from RB92579, RB975375, RB006629, RB011549 and RB064125 clones displayed elevated contents of total soluble carbohydrate even under water limitation (Fig. 7A). Nonetheless, only the clones RB006629, RB92579, and RB064125 displayed elevated photosynthesis rates (Fig. 3A), a response closely related to the best performance under water deficit situations. Thus, our findings suggest that carbohydrates accumulation was mostly associated to mechanisms to prevent water loss (osmotic adjustment) rather than for plant growth, as previously reported by Hayat et al. (2012), Santos et al. (2015) and Liu et al. (2019).

The starch consumption is assumed as an initial response to drought, allowing the maintenance of carbon supply in a condition of reduced atmospheric CO_2 fixation (Sreeharsha et al., 2019). For sugarcane, approximately 60% from total carbohydrates in RB867515 and RB955977 clones at 150 DAP (drought) was found to be starch; whereas similar values of total soluble carbohydrates and starch concentrations were noticed for other sugarcane clones (Fig. 7). This regulation suggests that starch hydrolysis was activated to maintain soluble carbohydrates levels in the clones RB92579, RB006629, RB975375, RB011549, and RB064125. Additionally, the higher starch content in RB867515 and RB955977 clones indicates a metabolic limitation caused by water deficit, once net photosynthesis was negatively influenced by water deficit (Fig. 7).

The stalk and sugar yield were superior in promising clones when compared to commercial clones, except to RB006629 sugarcane clone (Table 2). In general, the sugarcane-promising clones (except RB006629) displayed stalk and sugar yield around 70% superior to commercial clones. For all cases, the elevated productivity was closely related to photosynthetic performance, growth and biochemical adjustment, highlighting the potential clones as the most suitable to grown in environments with water limitation (Figs 3,6 and 7) (Sedigheh et al., 2011; Chumphu et al., 2019).

5. Conclusion

The promising sugarcane clones, mainly RB975375, RB006629, RB064109, and RB064125, exhibited better growth, gas exchange and osmotic responses under conditions of water limitations. Of these clones, the RB975375 and RB064109 displays better technological traits and higher stalk and sugar yields and therefore were more efficient in tolerating the water limitation when compared to commercial sugarcane clones.

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Figure legends

Figure 1. Rainfall, evapotranspiration of reference and air temperature (minimum, average and maximum) throughout the sugarcane crop cycle (April 2017 to June 2018). (Excel 2010)

Figure 2. Temporal variation of soil moisture at depths 0.1 to 0.6 m along the sugarcane crop cycle. Phase I = Sprouting. Phase II = Tillering. Phase III = Vegetative growth. Phase IV = Maturation. FC = Field capacity. PWP = Permanent withering point. HC = Critical humidity. (SigmaPlot 12.0)

Figure 3. CO_2 assimilation rate (A), transpiration rate (B), stomatal conductance (C) and internal CO_2 concentration (D) of commercial (RB867515 and RB92579) and promising sugarcane clones at 90 days after planting (under drought). Different capital letters represent significant difference between sugarcane clones (Scott Knott' test; $p < 0.05$). (SigmaPlot 12.0).

Figure 4. Instantaneous water-use efficiency (A), intrinsic water use efficiency (B) and instantaneous carboxylation efficiency (C) of commercial (RB867515 and RB92579) and promising sugarcane clones at 90 days after planting (under drought). Different capital letters represent significant difference between sugarcane clones (Scott Knott' test; $p < 0.05$). (SigmaPlot 12.0).

Figure 5. Principal component analyses with gas exchange variables measured at 90 days after planting in commercial (RB867515 and RB92579) and promising sugarcane clones. The vectors indicate the direction and strength of each analyzed variable for the general distribution. A = CO_2 assimilation rate. T = transpiration rate. gs = stomatal conductance. Ci = internal CO_2 content. A/E = instantaneous water-use efficiency. A/gs = intrinsic water use efficiency. A/Ci = instantaneous carboxylation efficiency. (Stat. 2004)

Figure 6. Plant height (A), stem diameter (B) and leaf area index (C) of commercial (RB867515 and RB92579) and promising clones of sugarcane plants evaluated at 90 and 150 days after planting (DAP). Different lowercase and capital letters represent significant difference between sugarcane clones at 60 and 150 DAP, respectively (Scott Knott' test; $p < 0.05$). (SigmaPlot 12.0).

Figure 7. Total soluble carbohydrates (A), starch (B) and free proline (C) of commercial (RB867515 and RB92579) and promising clones of sugarcane plants evaluated at 60 DAP (before the water deprivation) and 150 DAP (in drought). Different lowercase and capital letters represent significant difference between sugarcane clones at 60 and 150 DAP, respectively (Scott Knott' test; $p < 0.05$). DAP = Days after planting. (SigmaPlot 12.0).

Figure 8. Principal component analyses with biometric and biochemical parameters at 150 days after planting in commercial (RB867515 and RB92579) and promising sugarcane clones. The vectors indicate the direction and strength of each analyzed variable for the general distribution. PH = plant height. SD = stem diameter. LAI = leaf area index. TSC = total soluble carbohydrates. ST = starch. PR = free proline. (Stat. 2004)

Table 1. Chemical and physical attributes of soil samples at different depths collected in the experimental area before the developing of the experiment

		Depths (m)		
		0.0 - 0.2	**0.2 - 0.4**	**0.4 - 0.6**
Chemical Attributes	pH ($CaCl_2$)	6.5	5.1	7.2
	Organic matter (g dm^{-3})	0.9	0.8	0.6
	Phosphorus available (mg dm^{-3})	8.1	4.1	1.5
	K ($cmol_c$ dm^{-3})	11,4	13,4	0,1
	Ca ($cmol_c$ dm^{-3})	1.5	1.0	0.6
	Mg ($cmol_c$ dm^{-3})	0.5	0.3	0.3
	H + Al ($cmol_c$ dm^{-3})	0.7	1.2	1.1
	Sum of bases ($cmol_c$ dm^{-3})	2.0	1.4	0.9
	Capacity of cation exchange ($cmol_c$ dm^{-3})	2.7	2.6	2.0
	Saturation for bases (%)	74.2	53.7	45.9
Physical attributes	Clay (%)	10.6	10.7	15.6
	Silt (%)	2.8	2.8	0.3
	Sand (%)	86.7	86.5	84.2

Table 2. Technological quality traits and yields of sugarcane clones at the end of experiment (420 days after planting)

Clones	Technological Quality Traits				Yield	
	Apparent purity	Sucrose content	Fiber contents	Total recoverable sugar	Stalk	Sugar
		(%)		(Mg ha^{-1})	(Mg ha^{-1})	
RB867515	79.69 a	13.37 a	13.58 bc	136.79 ab	59.38 b	7.93 c
RB92579	79.36 a	13.53 a	14.93 ab	138.31 ab	62.50 b	8.45 c
RB975375	83.02 a	15.30 a	12.22 c	152.54 a	111.61 a	17.08 a
RB006629	78.52 a	13.89 a	13.91 bc	142.53 ab	57.59 b	7.99 c
RB955977	79.36 a	13.44 a	13.87 bc	137.73 ab	100.00 a	13.44 b
RB011549	78.89 a	12.97 a	13.76 bc	133.71 ab	102.68 a	13.32 b
RB064109	84.04 a	12.04 a	15.84 a	120.77 b	99.11a	11.93 b
RB064125	82.40 a	13.45 a	14.53 ab	135.37 ab	97.02 a	13.05 b

In columns, different lowercase letters represent significant difference between sugarcane clones according to Scott Knott' test ($p < 0.05$).

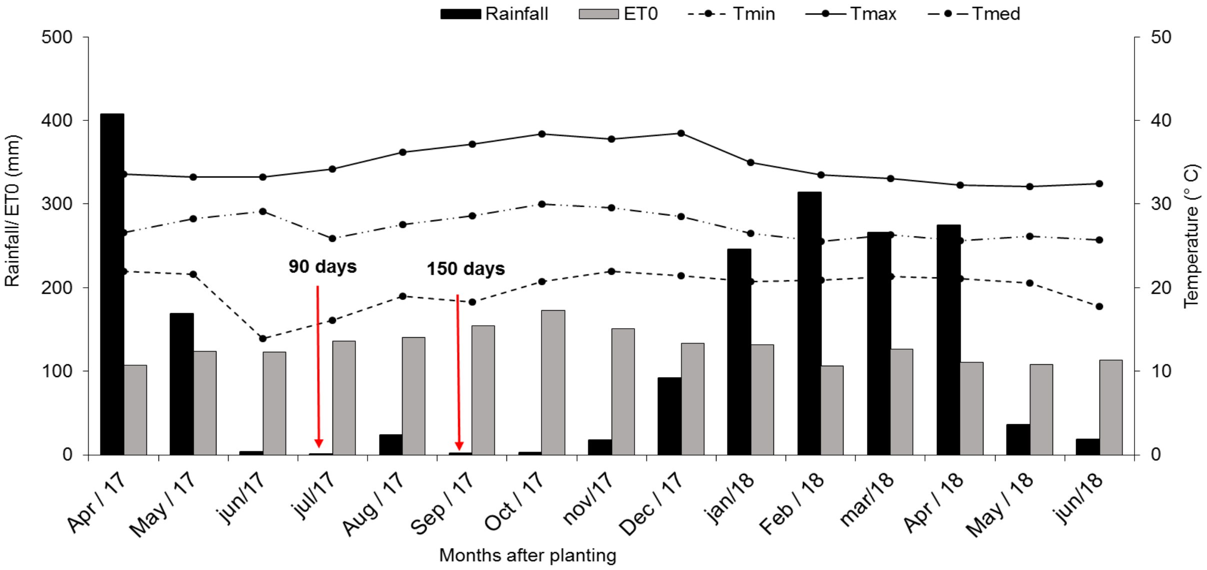
Rainfall
ET0
Tmin
Tmax
Tmed
Rainfall/ ET0 (mm)
Temperature (° C)
500
400
300
200
100
0
50
40
30
20
10
0
90 days
150 days
Apr / 17
May / 17
jun/17
jul/17
Aug / 17
Sep / 17
Oct / 17
nov/17
Dec / 17
jan/18
Feb / 18
mar/18
Apr / 18
May / 18
jun/18
Months after planting

Fig. 1.

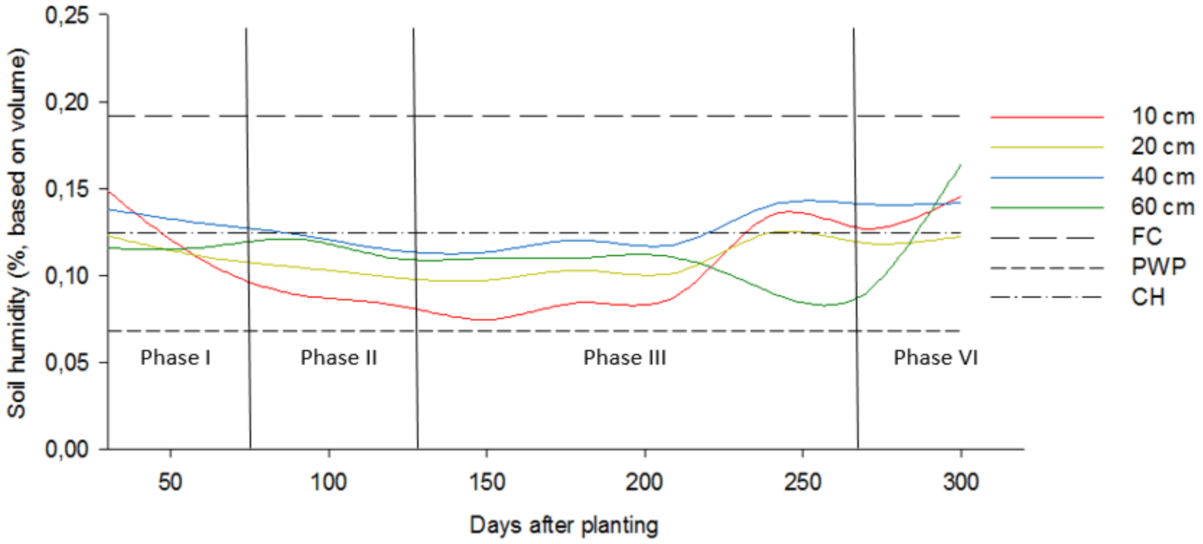
0,25
0,20
0,15
0,10
0,05
0,00
Soil humidity (%, based on volume)
50
100
150
200
250
300
Days after planting
Phase I
Phase II
Phase III
Phase VI
10 cm
20 cm
40 cm
60 cm
FC
PWP
CH

Fig. 2.

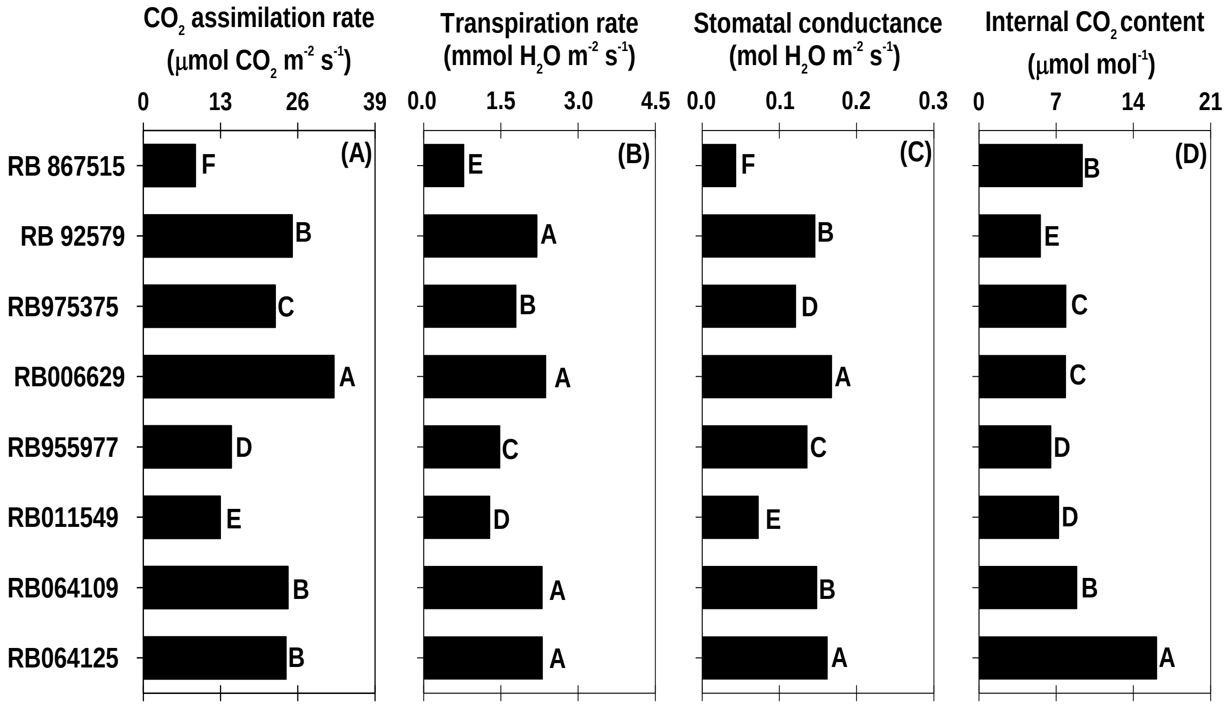
CO2 assimilation rate (μmol CO2 m-2 s-1)
0
13
26
39
(A)
Transpiration rate (mmol H2O m-2 s-1)
0.0
1.5
3.0
4.5
(B)
Stomatal conductance (mol H2O m-2 s-1)
0.0
0.1
0.2
0.3
(C)
Internal CO2 content (μmol mol-1)
0
7
14
21
(D)
RB 867515
RB 92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
F
B
C
A
D
E
B
B
E
A
B
A
C
D
A
A
F
B
D
A
C
E
B
A
B
E
C
C
D
D
B
A

Fig. 3.

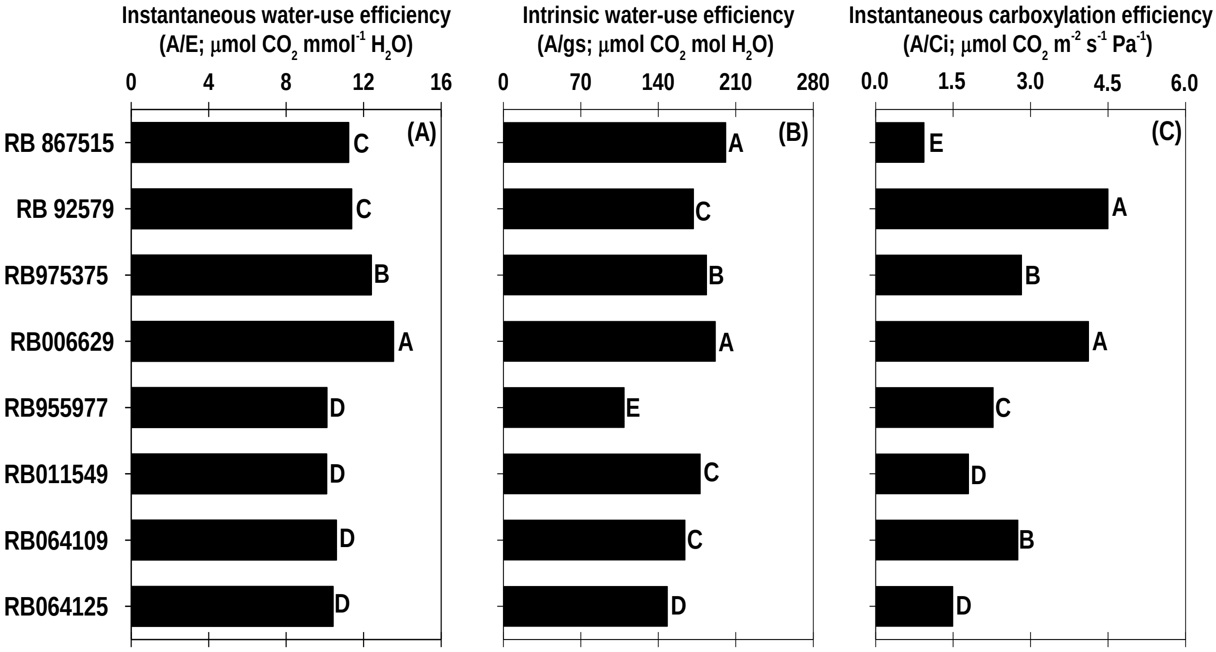
Instantaneous water-use efficiency
(A/E; µmol CO2 mmol-1 H2O)
0
4
8
12
16
Intrinsic water-use efficiency
(A/gs; µmol CO2 mol H2O)
0
70
140
210
280
Instantaneous carboxylation efficiency
(A/Ci; µmol CO2 m-2 s-1 Pa-1)
0.0
1.5
3.0
4.5
6.0
(A)
(B)
(C)
RB 867515
RB 92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
C
C
B
A
D
D
D
D
A
C
B
A
E
C
C
D
E
A
B
A
C
D
B
D

Fig. 4.

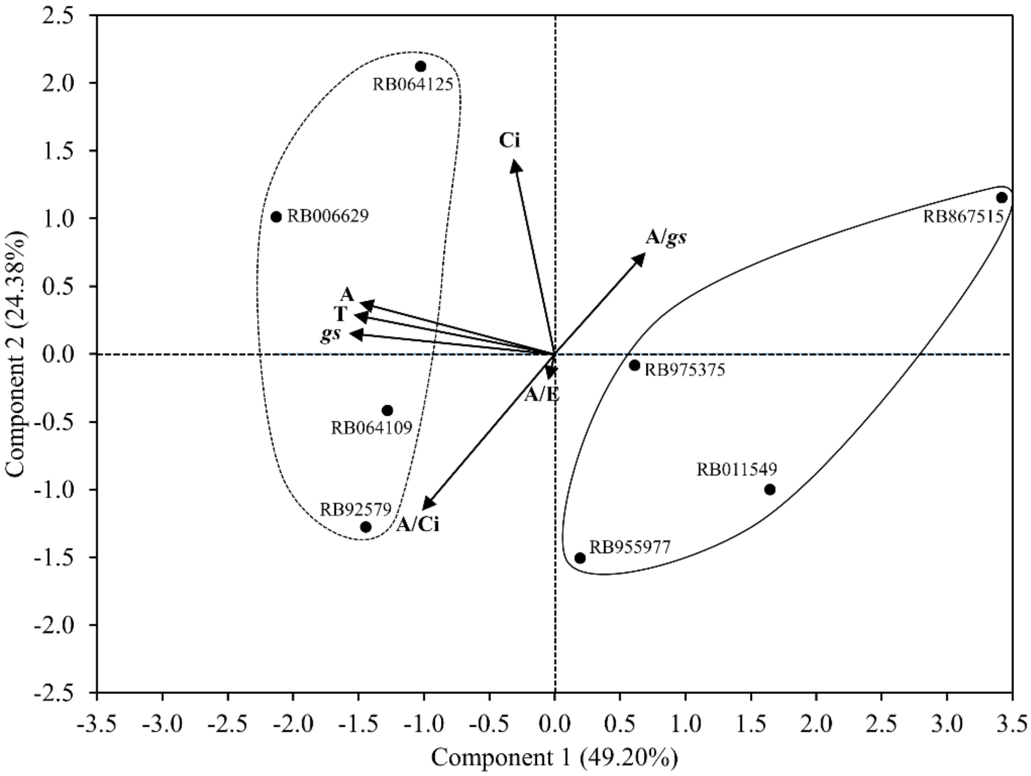
Component 2 (24.38%)
Component 1 (49.20%)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
RB064125
RB006629
RB064109
RB92579
RB867515
RB975375
RB011549
RB955977
Ci
A/gs
A
T
gs
A/E
A/Ci

Fig. 5.

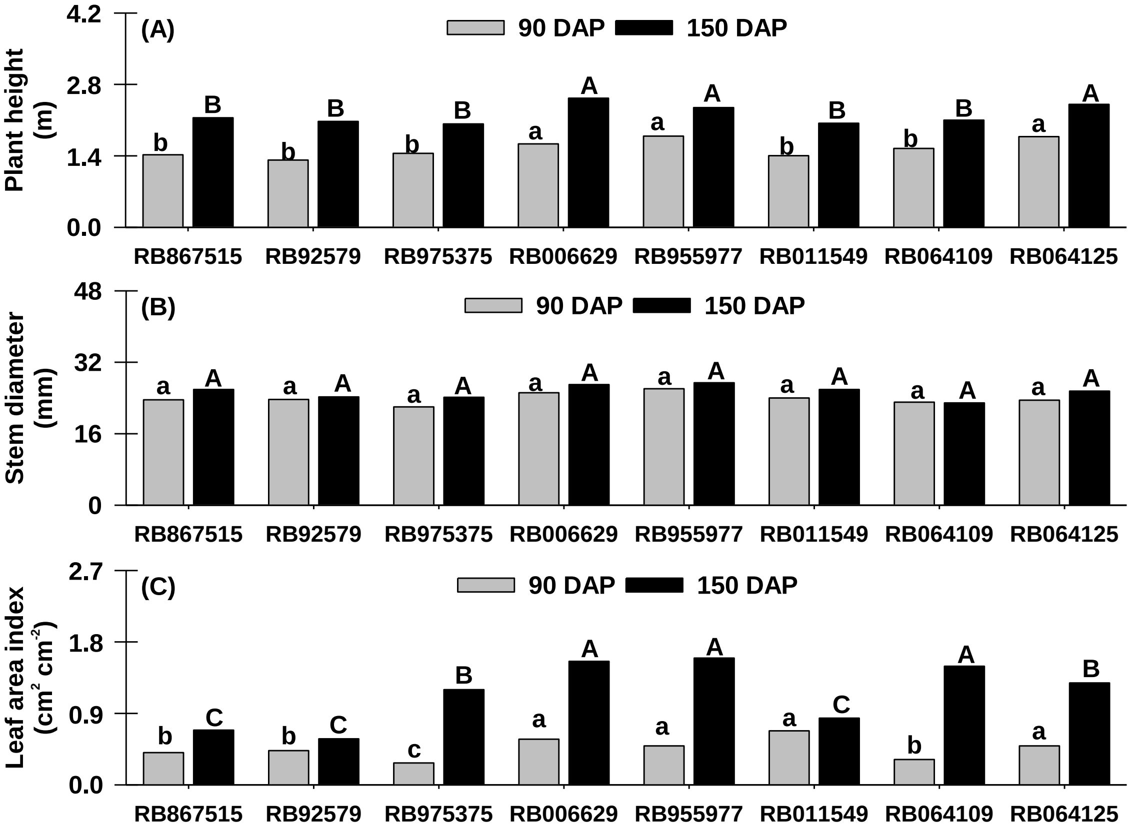
(A)
90 DAP
150 DAP
Plant height (m)
4.2
2.8
1.4
0.0
b
B
b
B
b
B
a
A
a
A
b
B
b
B
a
A
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
(B)
90 DAP
150 DAP
Stem diameter (mm)
48
32
16
0
a
A
a
A
a
A
a
A
a
A
a
A
a
A
a
A
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
(C)
90 DAP
150 DAP
Leaf area index (cm^2 cm^{-2})
2.7
1.8
0.9
0.0
b
C
b
C
c
B
a
A
a
A
a
C
b
A
a
B
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125

Fig. 6.

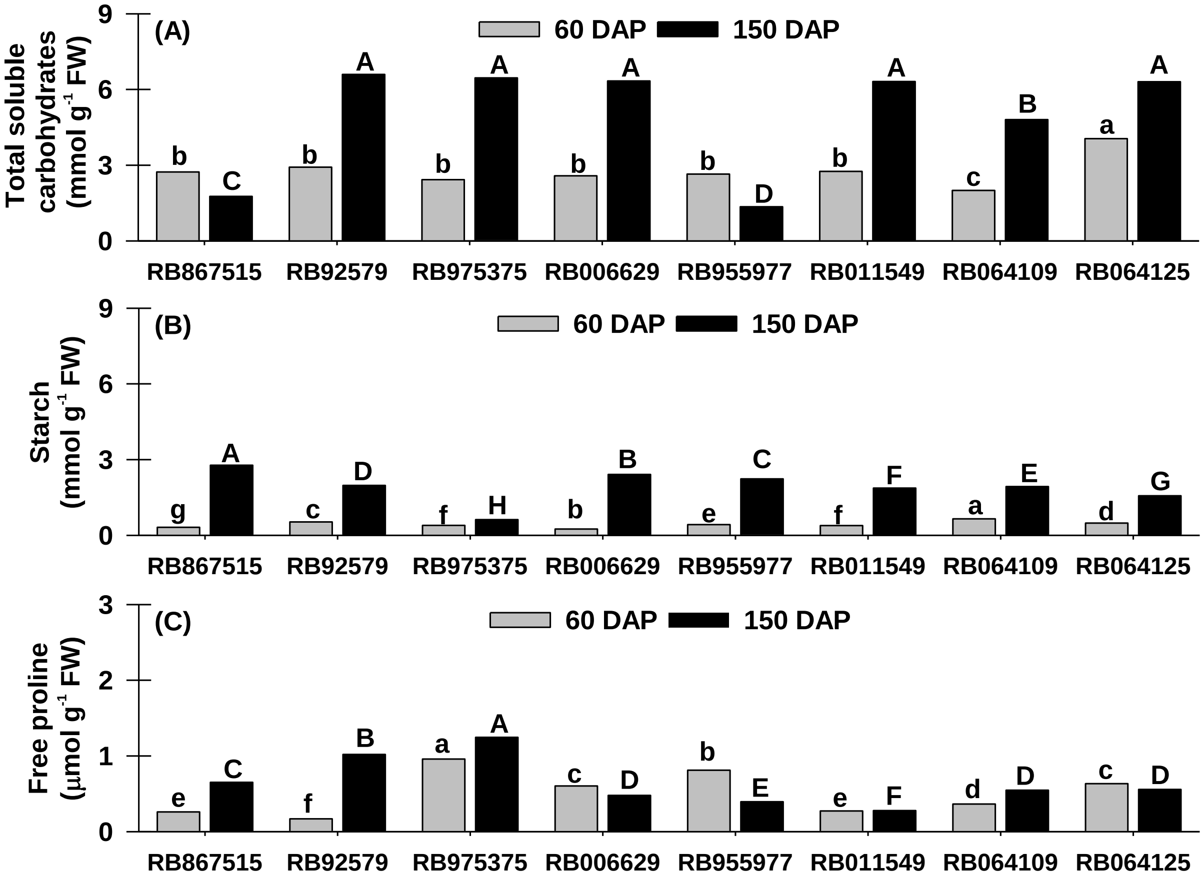
(A)
60 DAP
150 DAP
Total soluble carbohydrates (mmol g^{-1} FW)
9
6
3
0
b C
b A
b A
b A
b D
b A
c B
a A
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
(B)
60 DAP
150 DAP
Starch (mmol g^{-1} FW)
9
6
3
0
g A
c D
f H
b B
e C
f F
a E
d G
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125
(C)
60 DAP
150 DAP
Free proline (μmol g^{-1} FW)
3
2
1
0
e C
f B
a A
c D
b E
e F
d D
c D
RB867515
RB92579
RB975375
RB006629
RB955977
RB011549
RB064109
RB064125

Fig. 7.

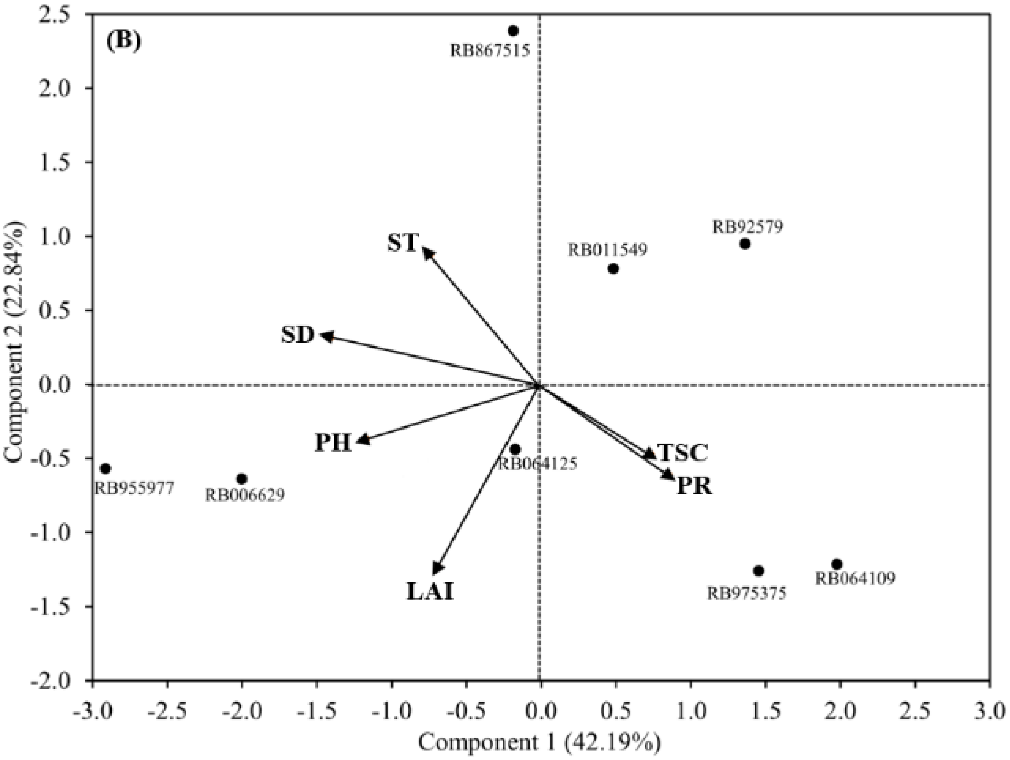
(B)
RB867515
ST
RB011549
RB92579
SD
PH
RB064125
TSC
PR
RB955977
RB006629
LAI
RB975375
RB064109
Component 2 (22.84%)
Component 1 (42.19%)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 8.

CAPÍTULO III

Osmoprotectants and technological yield in sugarcane clones grown in water-limited environments

(Artigo Submetido à Revista Plant Growth Regulation)

Artigo formatado de acordo com as normas do periódico

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ABSTRACT

Exposure to drought during vegetative development is considered the most limiting factor for sugarcane production. Accumulating compatible osmolytes under drought conditions constitutes an important trait to osmotic adjustment and the maintenance of cellular turgor under low water availability. The present study sought to evaluate the temporal variation of proline and carbohydrate accumulation in sugarcane plants grown under limited water conditions. The experiment was carried out in a randomized complete block design, consisting of six promising clones (RB975375, RB021754, RB955977, RB064125, RB006995, RB068027) and two commercial clones (RB867515 and RB92579), with three replications, totaling 24 plots. The assays were performed during two crop cycles (2017/2018 and 2018/2019 harvest) corresponding to planted sugarcane and one ratoon. Clones RB021754, RB064125, RB006995 and RB975375 exhibited higher carbohydrate contents in both evaluated periods (30 to 210 DAP), as compared to the commercial clones RB867515 and RB92579. Similarly, the clones RB021754, RB064125, RB068027, RB006995 and RB975375 exhibited a progressive increase in proline contents, indicating that both proline and carbohydrates may have acted in osmotic adjustment to reduce the leaf water potential. The increased accumulation of osmoregulators in the RB068027 and RB975375 clones for the two studied seasons were related to improved water use efficiency, even when water was scarce. In conclusion, the sugarcane clones display diversified biochemical characteristics indicating significant accumulation of osmoprotectors under water restriction in clones RB021754, RB064125, RB068027, RB006995 and RB975375, and clones RB068027 and RB975375 were highly efficient in water use, reflected in the best stalk and sugar yields.

Keywords: Dry season, Osmoregulators, Productivity, *Saccharum officinarum*, Water stress.

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1. INTRODUCTION

Sugarcane (*Saccharum spp.*) is an important component of the economy of many countries, where productivity tends to be lower in areas dependent on rainfall, a fact that has become more intense in recent years due to climate change as a result of less predictable rainfall distribution patterns (SANGHERA et al., 2019).

These factors, associated with high temperatures in many of these regions, result in the need for more research that will lead to the development of varieties adapted to low water availability (RAZA et al., 2019).

Plants may exhibit a series of responses when exposed to abiotic stresses, which may result in different degrees of tolerance or sensitivity to stress (ZANDALINAS et al., 2018). Under water deficit, the first metabolic changes are related to accumulation of osmoregulators, an attempt to reduce the water potential in plant cells and maintenance of the turgor pressure (HESSINI et al., 2009; MEDEIROS et al., 2013; NANDWAL et al., 2019). To balance the water potential between the cell's external environment, cytosol, and its organelles and vacuole, organic and non-toxic solutions are needed. Therefore, due to the accumulation made mainly by organic solutions that do not disturb the cellular metabolism (solutions used), they play an important role during acclimatization of plants to water and salt stresses (PRISCO et al., 2016).

Reduced leaf water potential mediated by accumulation of osmoregulators (also known as compatible solutes) is cited as a method ot protect cells against dehydration, and therefore is considered an important physiological acclimation to drought in plants (BASU et al., 2016; FAROOQ et al., 2017). Previous studies have demonstrated the role of osmoregulators in sugarcane and other species under restricted water conditions, highlighting the accumulation of proline and glycine betaine (SILVA et al., 2013; ZIVCAK et al., 2016; SANTOS et al., 2019; MAIA JÚNIOR et al., 2020). Also, compatible osmolytes such as glycine-betaine and free amino acids are necessary for plants to deal with the harmful effects of water-stress (MEDEIROS et al., 2013; ABBAS et al., 2014; SANTOS et al., 2015).

Osmotic adjustment constitutes one of several pathways underlying drought tolerance and others metabolic adjustments are crucial for plant performance under water deficit (MEDEIROS et al., 2012). Under water stress, excessive water loss by transpiration is avoided by reducing the stomatal conductance, which in turn limits CO_2 uptake and reduces CO_2 assimilation by Calvin's cycle during photosynthetic reactions (FAHAD et al., 2017). Consequently, plant growth and carbon fluxes are severely altered due to complications in metabolism and photo division assimilated in the whole plant which are responsible for inhibiting growth and reducing production (SANCHES et al., 2017).

Under high stress condition, when soluble sugars are used as osmoregulators, they may not be destined for the plant growth process. Thus, fluctuations in sugars are potentially the main cellular mechanisms against environmental variations, acting as substrates and modulators of enzyme activity and gene expression related to carbon and nitrogen metabolism (AINSWORTH; BUSH, 2011; BLUM, 2017).

Overall, physiological tools may facilitate the development of sugarcane clones adapted to stressful conditions and management techniques to cultivate to maintain yield under water stress conditions (QUEIROZ; SANTOS, 2009). In consideration of the above, this study sought to evaluate the temporal variation in carbohydrates and proline compounds, and their correlate with water use efficiency, total recoverable sugar levels and sugarcane productivity under water limited conditions.

2 - MATERIAL AND METHODS

2.1 – Experimental conditions

The study was conducted under a water stress conditions in a commercial sugarcane production area belonging to COMVAP Sugar and Alcohol LTDA, cultivated with sugarcane in previous years, in União, Piauí, Brazil (04°52'09" S, 42°52'45" W and elevation of 112 m). Before the test, soil samples from the experimental area were collected at three depths (0.0-0.2, 0.2-0.4 and 0.4-0.6 m) and submitted to chemical and particle size analysis (Tab. 1).

The region is characterized by a dry sub-humid climate, with excess rain from January to June and considerable water deficiency from July to December. In the two experimental cycles evaluated, the monthly air temperature and precipitation averages were obtained based on data from an automatic agrometeorological station at Embrapa Meio Norte (Teresina-PI) which is located in the experimental area.

The phenological development phases of the crop were organized according to the FAO recommendations described by Allen et al. (1998), with some modifications, considering the harvest of planted sugarcane after 14 months and harvest of the ratoon cane after 12 months (Table 2).

The experiment was setup in completely casualized blocks, with three repetitions, consisting of six promising sugarcane clones (RB975375, RB021754, RB955977, RB064125, RB006995, RB068027) from the Sugarcane Genetic Improvement Program of the Inter-University Network for the Development of the Sector Sucroenergetic (RIDESA) and two commercial varieties (RB867515 and RB92579) widely cultivated in Northeast Brazil, totaling 24 plots.

The assays were carried out in two consecutive harvests (2017/2018 and 2018/2019). During the first cycle (plant cane), planting was performed on April 10, 2017, and harvest on June 20, 2018, while harvest of the second cycle (ratoon cane) was conducted on June 27, 2019.

Each plot was composed of four 10.0 m long rows, with 1.40 m between rows, and the working area was defined as the two central rows, discarding 0.50 m at the end of each row to avoid the border effect. Planting was done manually, distributing six cane tillers with three buds per meter in the planting furrows, crossing foot with tip, totaling an average of 18 buds per meter of each clone per furrow. Before planting, fertilizing was performed according to the soil chemical analysis (Table 1), using 500 kg^{-1} of the 06-28-22 formulation at planting.

2.2- Crude extracts

For measure total soluble carbohydrates (TSC), sucrose (SUC) and starch (STAR), fresh samples from the diagnostic leaf (leaf +1) were collected at 30, 60, 150, 180 and 210 days after planting and cutting. Crude extracts were obtained by the MCW (methanol, chloroform and water) method, at 12:5:3 (v/v), as described by Bielesk and Turner (1966).

2.3 – Total soluble carbohydrates, sucrose and starch

Total soluble carbohydrates were measured by the sulfuric-carbolic acid method (DUBOIS et al., 1956); whereas sucrose and starch were measured according Van Handel (1968) and Amaral et al. (2007), respectively.

2.4 - Proline content

Proline was extracted and quantified at 90 and 150 days. The crude extract was obtained by macerating 1.0 g of fully expanded leaves (fresh material) using 5.0 mL of a 3% sulfosalicylic acid solution. The homogenate was centrifuged at 2000 × g and the proline was quantified by spectrophotometric readings at 520 nm using proline as a standard (Bates et al., 1973).

2.5 - Total recoverable sugar, stalks productivity and water use efficiency

Ten stalks from each sugarcane clone were collected within the working area. Total recoverable sugar (TRS, kg t^{-1}) and stalks yield per hectare (SYH, Mg ha^{-1}) were quantified according to technical standards of the Sugarcane, Sugar and Alcohol Producers Council of the State of Pernambuco (CONSECANA/PE, 2007). The water use efficiency (WUE) was obtained as the ratio between stalk productivity (Mg ha^{-1}) and total precipitation (m^3 ha^{-1}) and expressed in kg m^{-3}.

2.6 - Statistical analysis

To analyze the temporal variation of the carbohydrate concentration in the leaves of the sugarcane clones at 30, 60, 150, 180 and 210 days, the data were subjected to analysis of variance by the F-test at the level of 5% probability, and the means compared by the Tukey test. Means of the technological variables were compared using the Scott Knott test ($p \leq 0.05$). All statistical analyses were performed using the SISVAR® statistical program (FERREIRA, 2011).

3 - RESULTS

3.1 - Water availability in the soil and water use efficiency in sugarcane stems

Rainfall in both years was concentrated from December to April, ranging from 91.0 to 407.0 mm month^{-1}; while the lowest rainfall was registered from June to November, ranging from 18.0 to 23.6 mm month^{-1} (Fig. 1).

During cultivation of the plant cane (2017/18 harvest), the total precipitation was 1.890,80 mm (Fig. 1A), with the highest precipitation registered in April 2017. Contrarily, total precipitation in the ratoon cane (2018/19 harvest) was 1,589.20 mm, with low precipitation during seed germination and seedling establishment (40.7 mm), from June to November 2018 (Fig. 1A).

The mean air temperature varied between 25 and 33°C for both the 2017/18 and 2018/19 growing seasons. As a consequence of low rainfall and absence of clouds, there was a strong increase in ET0 and temperature, where the ET0 values were 2,095 (4.6 mm day^{-1}) and 1,390 mm (3.81 mm day^{-1}) in the 2017/2018 and 2018/2019, respectively. In the current study, soil moisture levels were reduced at depths below 0.2 m in the soil profile, to nearly critical moisture at 0.4-0.6 m (Fig. 2).

The cultivation coefficient (Kc) varied according to the water requirement at the different phenological stages (Fig. 3). It was higher in phase I of the plant cane compared to the same period of the ratoon cane, with higher water requirements in phases II and III due to the increase in tillers and the sugarcane growth.

Regarding water use efficiency, in the 2017/20018 season the clones RB068027 (6.59 kg m^{-3}), RB975375 (5.90 kg m^{-3}), RB955977 (5.29 kg m^{-3}) and RB064125 (5.13 kg m^{-3}) exhibited the highest water use efficiencies, whereas the clone RB867515 (3.14 kg m^{-3}) showed the lowest water use efficiency (Fig. 4). On the other hand, in the second season the clones RB975375 (7.50 kg m^{-3}) and RB068027 (6.19 kg m^{-3}) stood out as the most efficient with regards to water use compared to the other sugarcane clones. Contrarily, the commercial clones displayed the lowest water use efficiency values, being 1.40 kg m^{-3} for RB92579 and 2.31 kg m^{-3} for RB867515 (Fig. 4).

3.2 – Time-dependent alterations in carbohydrates and proline

After 30 days, accumulation of the compatible compounds varied with regards to both the growing season and evaluated clones (Fig. 5). In the first season (2017/2018), the clones RB021754 and RB006995 stood out with the highest proline contents, whereas carbohydrates and sucrose were found to be similar to the commercial clone RB92579; however the clone RB068027 exhibited the highest sucrose levels (Fig. 5A). On the other hand, in the second harvest (2018/2019) almost all clones displayed sucrose, carbohydrate and proline contents higher than the commercial clone RB92579, and the highest level was observed in clone RB021754. The starch content was similar for all studied clones, except RB068027.

In the 2017/2018 harvest, at 60 days the proline levels in all clones were lower than those of the commercial clone RB92579; and the highest sucrose and soluble carbohydrate contents were noted in the clones RB975375, RB 064125 and RB 068027 as compared to the commercial varieties (Fig. 5B). In the second harvest, the RB92579 clone showed the lowest values for all variables compared to the other evaluated sugarcane clones; and the clones RB021754, RB064125, RB006995 and RB068027 reached elevated osmolyte contents (Fig. 5B).

All sugarcane clones exhibited high carbohydrate and sucrose quantities compared to the commercial clone RB92579 at 150 days, and the highest proline levels were found in the clones RB006995 and RB975375 in the first harvest (2017/2018). Contrarily, in the 2018/2019 harvest significant alterations were noticed in starch and sucrose concentrations, with low proline accumulation in clones RB955977 and RB066995 compared to the commercial clone RB92579 (Fig. 5C).

The greatest variation in organic compounds in the 2017/2018 harvest was observed at 180 days, especially for soluble carbohydrates, and all sugarcane clones displayed greater accumulation than the commercial clone. The lone exception was the for RB006995 clone which showed lower carbohydrate, starch and sucrose values, and elevated proline levels in comparison to the reference clone (Fig. 5D). However, in the 2018/2019 season only the clones RB021754 and RB068027 exhibited greater soluble carbohydrate accumulation compared to the commercial clone RB92579.

At 210 days, in both seasons all parameters were significantly altered in the evaluated sugarcane plants. In the first season, the clones RB975375, RB021754, RB006995 and RB068027 stood out with higher proline and starch concentrations as compared to the commercial clone RB92579, except for clone RB975375 (Fig. 5E). In the second season, all sugarcane clones showed sucrose levels higher than the RB92579 clone, whereas little or no alteration was registered for proline levels. Clones RB 975375, RB 021754, RB955977 displayed soluble carbohydrate levels higher than the commercial clones.

Overall, by analyzing the accumulation of organic compounds in the two seasons, it was noted that clones RB975375, RB021754, RB955977, RB064125 and RB006995 displayed the highest sucrose levels, and the levels were higher as of 150 DAP in clones RB92579, RB975375, RB955977 and RB006995. However, the carbohydrate contents were higher in clones RB92579, RB975375, RB021754 and RB064125 at 150 DAP.

Starch accumulation took place mainly in clones RB92579, RB975375, RB021754, RB955977 and RB064125, with the highest values from 150 to 180 DAP (Fig. 5C and 5D). Additionally, the clones RB92579, RB975375 and RB064125 exhibited an increase in the proline contents mainly in the second season (at 150 DAP). Finally, the clones RB068027 and RB867515 exhibited lower levels of compatible osmolytes in both seasons, being most affected by water limitation during the evaluation period.

3.3 - Productivity and total recoverable sugar in sugarcane stems

The principal component analysis (PCA) was performed to analyze the distinction between sugarcane clones in relation to technological variables during cultivation of plant cane (2017/2018 season) and ratoon plant (2018/2019 season) (Fig. 6). In the first season there were similarities between the clones, evidenced by two well-defined groups, with a distinct influence on the characteristics analyzed which explains 70.82% and 19.24% of the total variation (Fig. 6A). In general, technological characteristics showed a strong association with the clones of groups I (RB064125, RB006995, RB955977, RB92579 and RB867515) and group II (RB068027, RB021754 and RB975375), constituting a positive indicator for selecting the most efficient clones to grow in a stressful environment.

The sugarcane Pol% (PCC), total recoverable sugar (TRS), apparent juice purity (PZA) and stalk yield per hectare (SYH) vectors exhibited a single direction and strong association with group II clones, revealing a low fiber content, but these variables showed no statistical difference for the clones (Table 3). Group I clones showed increased fiber production, but showed the opposite performance for PCC, TRS and PZA.

In the second harvest, the PCA exhibited the formation of two groups, the first (group I) formed by clones RB068027, RB867515, RB955977, RB006995, RB92579 and RB064125, and the second group (group II) formed by clones RB975375 and RB021754 (Fig. 6B). In the first component, the vectors Apparent purity, Sucrose content and Total recoverable sugar showed only one direction and a strong association with clones RB975375 and RB021754 (Fig. 6B).

The clones from group I showed a low correlation with most characteristics and the fiber variable was strongly associated with clone RB064125, but the opposite performance was noticed for the SYH variable.

Regarding the SYH, the clone RB068027 showed a strong association since these two vectors presented opposite performance. Thus, the technological characteristics explain 56.27% and 25.44% of the total variation (Fig. 6B).

4- DISCUSSION

4.1- Modulation of the carbohydrate metabolism in sugarcane clones grown in under water stress conditions

In the current study, clones RB021754, RB064125, RB006995 and RB975375 showed good performance under stressful conditions, with high carbohydrate contents from 30 to 210 DAP as compared to the commercial clones RB92579 and RB867515 (Fig. 5).

The increase in total soluble carbohydrates in plants subjected to water has been associated with physiological adjustment to reduce the osmotic potential to keep the tissues hydrated, thus delaying or avoiding cell and tissue dehydration (ZOUAOUI et al., 2019). This argument was further corroborated by Sales et al. (2012) in sugarcane plants under root stress conditions, who showed that the foliar content of non-structural carbohydrates, sucrose and starch, increased in plants under root cold.

In fact, a decrease in carbohydrates is closely associated with cell dehydration and reductions in starch content and photosynthesis in plants subjected to water stress (PATADE et al., 2011; SMITH et al., 2020). These complications imply low plant growth due to reductions in sucrose synthesis and transport, the main compound responsible for carbohydrate balance in the cell environment (SMITH et al., 2020).

Herein, a temporal variation in carbohydrate accumulation was registered from 30 to 210 DAP in both seasons (Fig. 7), but in the second season (at the beginning of development) the clones exhibited greater carbohydrate contents, which were higher than the commercial clones, correlating with the period of higher water restriction (Fig. 1).

During the intense growth phases (phase II and phase III) (Table 2), the period of low humidity (Fig. 2) as a result of minimal precipitation (Fig. 1), the carbohydrate accumulation was found to progressively increase in clones RB021754, RB064125, RB006995 and RB975375 (Fig. 5). These finding suggest that these clones are more effective in controlling stomatal conductance in water deficient soils compared to the other sugarcane clones (Table 3).

On the other hand, starch concentrations in the leaves were reduced due to lower carbon assimilation in sugarcane clones under water deficit, as observed in clones RB068027 and RB867515 (Fig. 5). It is possible that the reduction of photosynthesis decreased the concentration of triose phosphate, glucose and fructose phosphate in the leaf, essential substrates for the synthesis of sucrose and starch (DU et.al., 1998). Thus, data from the present study indicates that leaf starch accumulation might be an important indicator of tolerance to water deficit in sugarcane plants, acting as an energy source and a carbon skeleton for osmoregulation purposes (THALMANN et al., 2016).

4.2 – The role of proline accumulation to alleviate water deficit effects in sugarcane clones under water stress conditions

Proline may display numerous physiological roles in plant cells, including cytosol, chloroplasts, and mitochondria, in order to protect cell components and allow metabolic pathways (KAUR and ASTHIR 2015). It may act in maintaining the integrity of chloroplast structures by minimizing the damage to the PSII photochemical mechanisms caused by water deficit, as well protecting against oxidative damage to chloroplasts, mitochondria and proteins (GUIDI et al., 2019). Furthermore, proline may be an important osmoregulator in response to drought, as previously reported in soybean (LOCKE and ORT'S 2014) and maize plants (CHEN et al., 2016).

In this study, the clones RB92579, RB975375, RB064125 and RB006995 exhibited a progressive increase in proline content throughout the experiment, a response possibly activated to decrease the leaf water potential under water restriction (Fig 5). This was further corroborated by Santos et al. (2019), who showed a close relationship between an elevated proline content and decreased osmotic potential in the sugarcane varieties RB855536 and RB855113 submitted to water stress. Similarly, Santos and Silva (2015) reported the beneficial role of proline accumulation in water-stressed plants, highlighting its association with drought tolerance in sugarcane crop.

On the contrary, the clones RB021754, RB955977, RB068027 and RB867515 showed low proline levels in both crop seasons, with decreases over time (30, 60,150, 180 and 210 DAP and DAC) (Fig 5). The low accumulation of proline (a potent osmoregulator under stress) suggests that other mechanisms could be activated in response to drought, like a larger root system, taller stem and greater number of tillers (ENDRES et al., 2018), permitting that the plants avoid the harmful drought effects and exhibit good yields even under water stress.

4.3. The accumulation of osmoprotectors as an essential mechanism of tolerance to water deficit and productivity in sugarcane plants

In the present study, clones RB021754, RB064125, RB006995 and RB975375 exhibited high carbohydrate contents over time as of 60 DAP even under strong water limitation in the second season, while clones RB068027, RB867515 and RB955977 exhibited little or no variation, with low values (Fig 5). Low carbohydrate accumulation may arise from both low CO_2 assimilation by Calvin’s cycle and reduced starch accumulation in chloroplast during the day in water-stressed plants, because starch acts as a donor of carbon skeleton for the biosynthesis of soluble carbohydrates and proline in response to water deficiency (MACNEILL et al., 2017; THALMANN et al., 2017).

The clones RB975375, RB068027 and RB064125 displayed high sucrose levels, mainly at 210 days (Table 3 and Fig. 5), the time corresponding to technological evaluations of the first season. These findings indicate a superior performance of the RB975375, RB068027 and RB064125 clones, since sucrose accumulation constitutes the main stage of plant development and maturation, depending on physiological process for sugars biosynthesis and storage (WATT et al., 2014).

In plant cane cultivation, clones RB975375, RB068027, RB955977, and RB064125 stood out with the highest average stalk (RB068027) and sugar yields (RB975375 and RB068027), even when compared to the commercial clone RB92579, characterized as the most efficient in water use (Table 3 and Fig. 4). In the second season, clones RB975375 and RB068027 displayed superior performance among all studied sugarcane clones (Fig. 4), with the highest TRS and stalk yield values in clone RB975375 (Table 3), even under the highest water limitation from 30 to 120 DAP (Fig. 1).

5 - CONCLUSION

In conclusion, the sugarcane clones RB021754, RB064125, RB068027, RB006995 and RB975375 present expressive accumulation of osmoprotectors under water restriction. The clones RB068027 and RB975375 showed high water use efficiency in both the plant cane and ratoon cane cycles, in addition to higher sugar and stalk yield, and emerge as potential clones for cultivation in water limited soils.

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Figure legends

Figure 1. Average, maximum and minimum temperatures (°C), precipitation (mm) and reference evapotranspiration (ET0), on a monthly scale, during two sugarcane crop cycles (plant cane and ratoon).

Figure 2. Temporal variation of soil moisture at depths of 0.10 to 0.60 m during the sugarcane crop cycle and the sugarcane developmental phases. (FC - Field capacity; PWP - Permanent withering point and CH - Critical humidity).

Figure 3. Main crop coefficients in the vegetative phases in the 2017/2018 season (plant cane) and 2018/2019 season (ratoon cane).

Figure 4. Water use efficiency (WUE) of sugarcane clones under water stress in two seasons of cultivation, corresponding to cane plant (year 1) and first ratoon (year 2).

Figure 5. Grouping analysis of proline (Prol), starch (Star), soluble carbohydrates (Carb) and sucrose (Suc) in sugarcane leaves under water-stress conditions at 30 days (A) and 60 days (B), during plant cane (2017/2018 season) and ratoon (2018/2019 season). Each row represents one parameter (Prol, Star, Carb, Suc) and each column represents a sugarcane clone. The data were normalized to the commercial clone RB92579 as the reference plant, indicated as a green color. Relative changes are indicated as increasing the color intensity of the relative compound levels from low to high

Figure 6. Principal Component Analysis (PCA) with multivariate variation between sugarcane clones and technological characteristics in the 2017/2018 season (plant cane, A) and 2018/2019 season (ratoon cane, B). The vectors indicate the direction and strength of each variable analyzed for general distribution. Total recoverable sugar (TRS), Fiber% (F), Stalks yield per hectare (SYH), Sucrose contents in the cane - pol% cane (PCC), Apparent juice purity (PZA).

Table 1. Chemical and granulometric characteristics of the soil in the experimental area at different depths (0.0-0.2, 0.2-0.4 and 0.4-0.6 m)

	Depth (m)				**Depth (m)**		
	0.0-0.2	**0.2-0.4**	**0.4-0.6**		**0.0-0.2**	**0.2-0.4**	**0.4-0.6**
			Chemical characteristics				
pH ($CaCl_2$)	6.5	5.1	7.2	**Mg (cmolc dm^{-3})**	0.5	0.3	0.3
MO (%)	0.9	0.8	0.6	**H + Al (cmolc dm^{-3})**	0.7	1.2	1.1
$P_{disp.}$ (mg dm^{-3})	8.1	4.1	1.5	**SB (cmolc dm^{-3})**	2.0	1.4	0.9
K (mg dm^{-3})	11.4	13.4	0.1	**T (cmolc dm^{-3})**	2.7	2.6	2.0
Ca (cmolc dm^{-3})	1.5	1.0	0.6	**V (%)**	74.2	53.7	45.9
			Chemical granulometric				
Sand (g dm^{-3})	86.7	86.5	84.2	**Clay (g dm^{-3})**	10.6	10.7	15.6
Silt (g dm^{-3})	2.8	2.8	0.3				

Table 2. Characterization of phenological phases of sugarcane cultivation in the 2017/2018 (plant cane) and 2018/2019 (ratoon cane) harvest

Plant cane (2017/2018 season)

Phenological phases (Characteristic morphophysiological)		Period	Days after planting (DAP)	Duration (Days)
Phase I	Sprouting and establishment	10/04/17 to 10/06/17	0 to 60	60
Phase II	Profiling and growth	11/06/17 to 10/10/17	61 to178	118
Phase III	Maximum development	11/10/17 to 10/04/18	179 to 360	182
Phase IV	Maturation	11/04/18 to 26/06/18	361 to 420	137
Total Cycle		10/04/17 to 26/06/18	-	420

Ratoon cane (2018/2019 season)

Phenological phases (Characteristic morphophysiological)		Period	Days after Cut (DAC)	Duration (Days)
Phase I	Sprouting and establishment	26/06/18 to 26/08/18	0 to 60	60
Phase II	Profiling and growth	27/08/18 to 26/12/18	61 to178	118
Phase III	Maximum development	27/12/18 to 26/04/19	179 to 300	127
Phase IV	Maturation	27/04/19 to 27/06/19	300 to 360	60
Total Cycle		26/06/18 to 27/06/19	-	365

Table 3. Technological quality and productivity of sugarcane clones under water stress conditions in two cultivation seasons, corresponding to plant cane (2017/2018 season) and ratoon (2018/2019 season). The assays of apparent purity, sucrose contents in the cane - pol% cane, fiber content, total recoverable sugar, stalk and sugar were measured at 420 days after planting and 365 and days after cutting

	Plant cane (2017/2018 season)					
Clones	**Technological Quality Traits**				**Yield**	
	Apparent purity	Sucrose content	Fiber contents	Total recoverable sugar	Stalk	Sugar
		(%)		Mg ha^{-1}	Mg ha^{-1}	
RB92579	79.36 a	13.53 a	14.93 a	138.31 a	87.06d	11.80 b
RB867515	79.68 a	13.36 a	13.58 ab	136.79 a	59.40f	7.93 c
RB975375	83.02 a	15.29 a	12.22 b	152.54 a	111.60b	17.06 a
RB021754	84.75 a	14.59 a	13.95 ab	144.43 a	70.10e	10.26 c
RB955977	79.35 a	13.44 a	13.86 ab	137.72 a	100.00c	13.36 b
RB064125	82.40 a	13.45 a	14.52 ab	135.36 a	97.00c	13.06 b
RB006995	78.14 a	12.66 a	15.88 a	130.88 a	81.26d	10.36 c
RB068027	84.83 a	14.40 a	14.28 ab	142.53 a	124.56a	17.93 a

	Ratoon cane (2018/2019 season)					
Clones	**Technological Quality Traits**				**Yield**	
	Apparent purity	Sucrose content	Fiber contents	Total recoverable sugar	Stalk	Sugar
		(%)		Mg ha^{-1}	Mg ha^{-1}	
RB92579	80.33 a	14.42 a	13.55 a	141.54 b	22.30 d	3.23 d
RB867515	78.34 a	13.77 a	13.26 a	134.22 b	36.70 d	5.00 d
RB975375	81.98 a	15.48 a	12.84 a	153.02 a	119.20 a	18.53 a
RB021754	84.20 a	15.56 a	13.57 a	155.65 a	56.20 c	8.70 c
RB955977	81.23 a	14.45 a	13.07 a	141.97 b	46.00 c	6.63 c
RB064125	80.48 a	13.47 a	14.24 a	132.96 b	62.46 c	8.40 c
RB006995	81.88 a	14.17 a	13.77 a	140.76 b	48.03 c	6.83 c
RB068027	81.47 a	13.66 a	13.38 a	135.66 b	98.36 b	13.43 b

Table 4. Sucrose, carbohydrates, starch, and proline contents in the shoot of sugarcane clones grown under hydric stress, evaluated to 30, 60, 150, 180 and 210 DAP and DAC) in plant cane (2017/2018 season) and ratoon cane (2018/2019 season)

	Plant cane (2017/2018 harvest)				Ratoon cane (2018/2019 harvest)			
30 days	**Suc. 1**	**Carb. 1**	**Star. 1**	**Prol. 1**	**Suc. 2**	**Carb. 2**	**Star. 2**	**Prol. 2**
RB92579	196,1^{ab}	2340,5^{a}	1539,1^{a}	0,65^{c}	124,9^{g}	1018,0^{g}	1282,5^{a}	0,73^{c}
RB975375	182,3^{ab}	1997,3^{b}	1508,7^{a}	0,46^{e}	131,7^{f}	1158,0^{e}	1257,3^{b}	0,67^{c}
RB021754	162,2^{b}	2158,4^{ab}	835,7^{d}	1,08^{a}	222,2^{b}	1765,0^{b}	696,4^{e}	1,11^{a}
RB955977	142,6^{b}	2219,9^{ab}	696,4^{e}	0,55^{de}	86,4^{h}	2323,9^{a}	580,3^{h}	1,02^{a}
RB064125	157,3^{b}	2413,6^{a}	707,3^{e}	0,57^{cd}	213,6^{c}	1023,2^{f}	589,4^{g}	0,88^{b}
RB006995	152,4^{b}	2259,6^{ab}	1357,0^{b}	0,76^{b}	180,4^{d}	1569,8^{d}	980,7^{c}	1,02^{a}
RB068027	387,7^{a}	2394,2^{a}	944,6^{c}	0,58^{cd}	249,3^{a}	1738,4^{c}	846,2^{d}	0,66^{c}
RB 867515	109,9^{b}	2255,7^{ab}	785,9^{d}	0,32^{f}	152,0^{e}	723,6^{h}	654,9^{f}	0,88^{b}
60 days	**Suc. 1**	**Carb. 1**	**Star. 1**	**Prol. 1**	**Suc. 2**	**Carb. 2**	**Star. 2**	**Prol. 2**
RB92579	157,8^{d}	2924,8^{c}	526,0^{c}	0,16^{d}	129,6^{f}	954,5^{g}	438,3^{bc}	0,60cd
RB975375	238,8^{c}	2430,7^{d}	393,7^{f}	0,96^{a}	138,4^{e}	1278,7^{d}	328,1^{e}	0,50d
RB021754	177,9^{d}	2472,0^{d}	315,1^{g}	0,54^{c}	223,9^{a}	1701,4^{a}	705,7^{a}	0,93b
RB955977	161,3^{d}	2648,6^{cd}	421,7^{e}	0,81^{b}	130,7^{f}	1152,2^{e}	351,4^{de}	0,55cd
RB064125	551,8^{a}	4049,9^{b}	483,2^{d}	0,63^{c}	171,3^{d}	1473,8^{c}	402,7^{cd}	1,28a
RB006995	244,4^{c}	1669,8^{e}	545,4^{b}	1,00^{a}	139,1^{e}	726,2^{h}	454,5^{bc}	1,37a
RB068027	478,8^{b}	6406,0^{a}	575,0^{a}	0,92^{ab}	199,1^{b}	1620,4^{b}	479,2^{b}	1,02b
RB 867515	161,8^{d}	2732,6^{cd}	315,1^{g}	0,26^{d}	178,8^{c}	1037,4^{f}	262,6^{f}	0,65c
150 days	**Suc. 1**	**Carb. 1**	**Star. 1**	**Prol. 1**	**Suc. 2**	**Carb. 2**	**Star. 2**	**Prol. 2**
RB92579	198,6^{e}	6598,1^{a}	1977,9^{c}	1,02^{c}	479,2 ^{d}	967,4 ^{ef}	1648,2^{c}	1,59^{a}
RB975375	268,0^{c}	6455,0^{a}	618,6^{d}	1,25^{b}	205,6 ^{g}	1537,4^{b}	515,5^{f}	1,14^{c}
RB1754	314,9^{b}	5868,3^{b}	2230,8^{b}	0,49^{ef}	338,9 ^{e}	1298,1^{c}	1859,0^{b}	1,32^{b}
RB955977	437,4^{a}	1349,2^{d}	2236,2^{b}	0,40^{f}	497,9 ^{cd}	850,7 ^{fg}	1863,5^{b}	0,86^{ef}
RB064125	164,8^{f}	6305,6^{ab}	1567,1^{d}	0,56^{de}	243,7 ^{f}	2157,9^{a}	1305,9^{d}	1,11^{cd}
RB006995	307,7^{c}	6190,0^{ab}	2329,8^{b}	2,53^{a}	513,1 ^{c}	1012,8^{de}	1940,7^{b}	0,79^{f}
RB068027	225,7^{d}	3298,3^{c}	1880,6^{c}	0,94^{d}	668,5 ^{a}	1132,8^{d}	1041,3^{e}	0,87^{e}
RB 867515	111,3^{g}	1767,8^{d}	2778,5^{a}	0,65^{d}	623,9 ^{b}	737,2 ^{g}	2315,4^{a}	1,06^{d}
180 days	**Suc. 1**	**Carb. 1**	**Star. 1**	**Prol. 1**	**Suc. 2**	**Carb. 2**	**Star. 2**	**Prol. 2**
RB92579	481,8^{e}	6647,9^{a}	1893,1^{cd}	0,33 ^{e}	481,8e	848,8^{f}	1577,6^{cd}	0,82^{c}
RB975375	605,7^{c}	6170,2^{b}	1802,0^{e}	0,13 ^{f}	605,7c	1310,4^{c}	1501,7^{d}	0,53^{e}
RB021754	1197,5^{a}	6725,8^{a}	1977,1^{cd}	0,56 ^{c}	1197,5a	1575,6^{b}	1647,6^{c}	0,82^{c}
RB955977	596,9^{d}	2271,2^{e}	2700,7^{a}	0,79 ^{a}	596,9d	990,1^{e}	2250,6^{a}	0,69^{ed}
RB064125	235,5^{h}	3733,2^{d}	1269,8^{f}	0,42 ^{d}	235,5h	1006,3^{e}	1058,2^{e}	0,92^{b}
RB006995	295,0^{g}	6588,0^{a}	1771,7^{e}	0,62 ^{b}	295,0g	701,6^{g}	1476,4^{d}	0,97^{b}
RB068027	427,9^{f}	5238,1^{c}	2186,4^{bc}	0,42 ^{d}	427,9f	1807,7^{a}	1530,9^{d}	0,73^{d}
RB 867515	653,6^{b}	1862,0^{f}	2317,9^{b}	0,46 ^{d}	653,6b	848,1^{f}	1931,6^{b}	1,09^{a}
210 days	**Suc. 1**	**Carb. 1**	**Star. 1**	**Prol. 1**	**Suc. 2**	**Carb. 2**	**Star. 2**	**Prol. 2**
RB92579	517,5^{b}	3288,2^{bc}	332,2^{d}	0,13^{f}	393,1^{h}	700,3^{b}	276,9^{d}	1,06^{b}
RB975375	605,5^{a}	6616,0^{a}	405,4^{c}	0,37^{ab}	825,6^{c}	243,8^{h}	336,5^{c}	0,56^{d}
RB021754	469,4^{c}	5619,3^{a}	945,4^{a}	0,29^{bc}	910,1^{c}	605,6^{c}	778,7^{a}	0,90^{c}
RB955977	110,4^{g}	6321,1^{a}	302,7^{de}	0,17^{f}	1019,1^{b}	962,2^{a}	252,2^{e}	1,10^{b}
RB 064125	338,5^{e}	2195,0^{c}	295,7^{de}	0,24^{e}	648,9^{f}	336,5^{g}	247,0^{ef}	1,05^{b}
RB006995	424,8^{d}	4020,3^{b}	612,4^{b}	0,25^{de}	737,6^{e}	508,3^{e}	510,3^{b}	1,09^{b}
RB068027	437,2^{cd}	2265,0^{c}	133,8^{f}	0,33^{bc}	455,6^{g}	545,3^{d}	143,9^{g}	1,33^{a}
RB 867515	180,20^{f}	6271,3^{a}	279,3^{e}	0,40^{a}	1279,4^{a}	409,8^{f}	232,8^{f}	0,86^{c}

Values followed by different lowercase letters in the same column represent a significant difference due to the sugarcane clone, according to the Tukey test ($p < 0.05$).

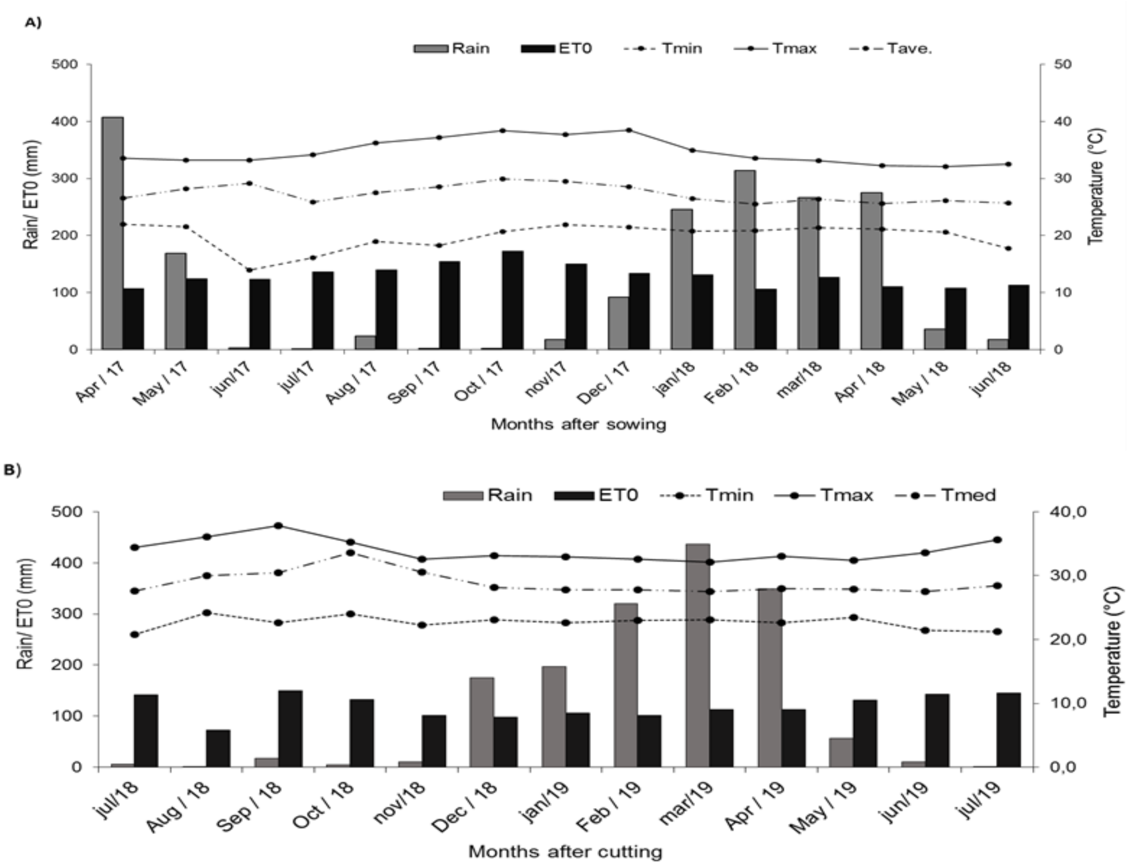
A)
Rain
ET0
Tmin
Tmax
Tave.
Rain/ ET0 (mm)
Temperature (°C)
500
400
300
200
100
0
50
40
30
20
10
0
Apr / 17
May / 17
jun/17
jul/17
Aug / 17
Sep / 17
Oct / 17
nov/17
Dec / 17
jan/18
Feb / 18
mar/18
Apr / 18
May / 18
jun/18
Months after sowing
B)
Rain
ET0
Tmin
Tmax
Tmed
Rain/ ET0 (mm)
Temperature (°C)
500
400
300
200
100
0
40,0
30,0
20,0
10,0
0,0
jul/18
Aug / 18
Sep / 18
Oct / 18
nov/18
Dec / 18
jan/19
Feb / 19
mar/19
Apr / 19
May / 19
jun/19
jul/19
Months after cutting

Figure 1.

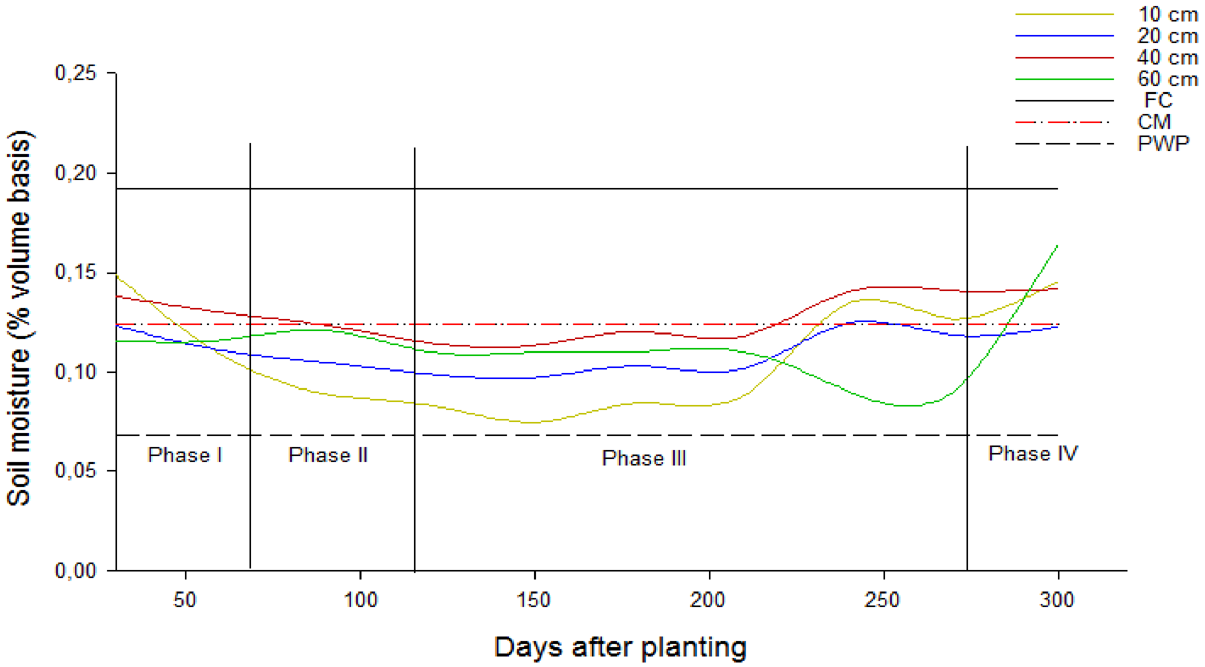
10 cm
20 cm
40 cm
60 cm
FC
CM
PWP
Soil moisture (% volume basis)
0,25
0,20
0,15
0,10
0,05
0,00
Phase I
Phase II
Phase III
Phase IV
50
100
150
200
250
300
Days after planting

Figure 2.

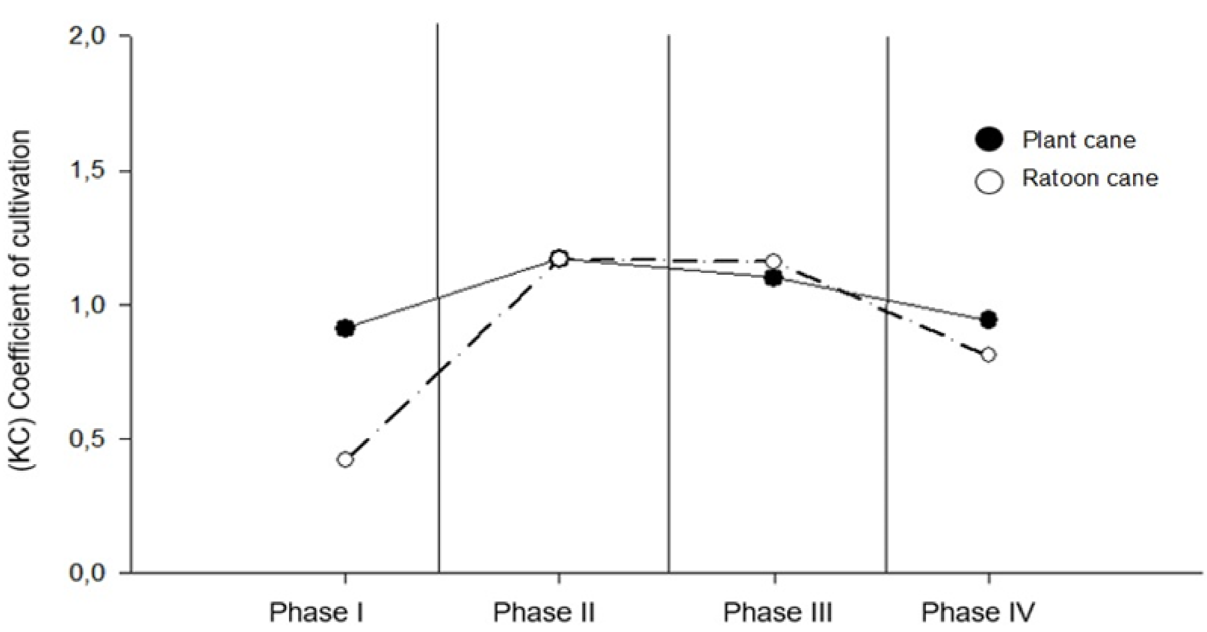
2,0
1,5
1,0
0,5
0,0
(KC) Coefficient of cultivation
● Plant cane
○ Ratoon cane
Phase I
Phase II
Phase III
Phase IV

Figure 3.

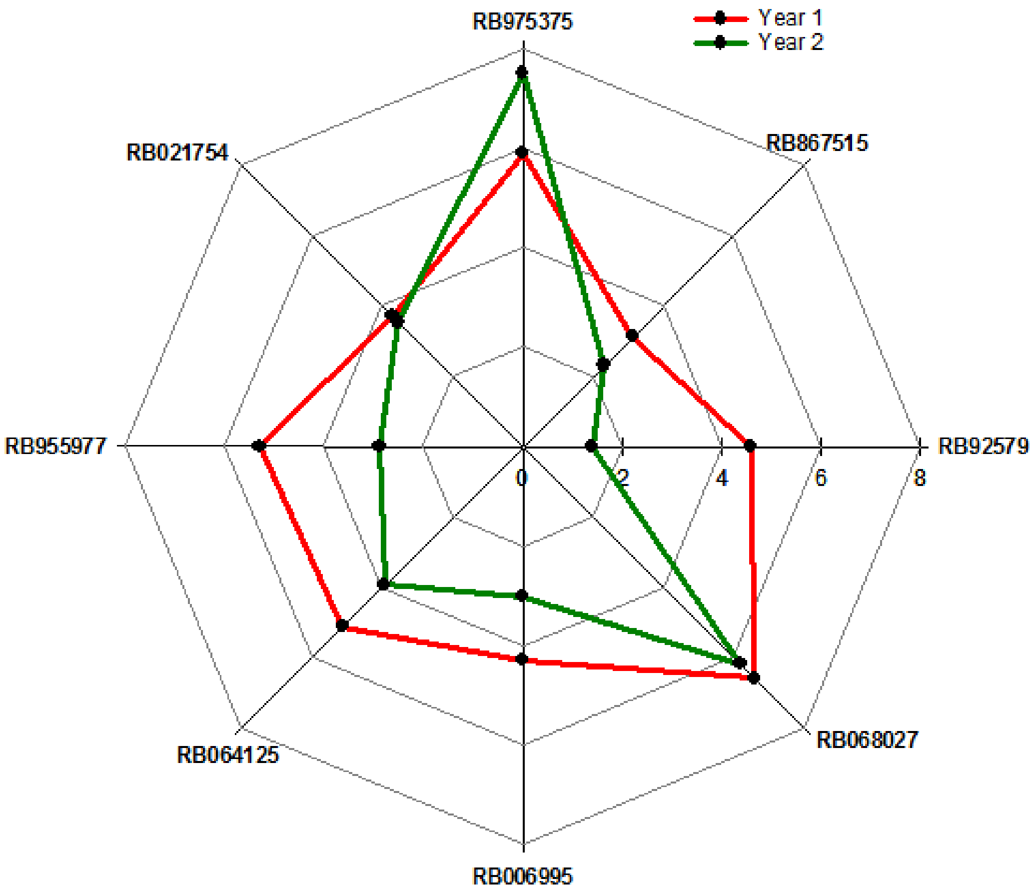
Year 1
Year 2
RB975375
RB867515
RB92579
RB068027
RB006995
RB064125
RB955977
RB021754
0
2
4
6
8

Figure 4.

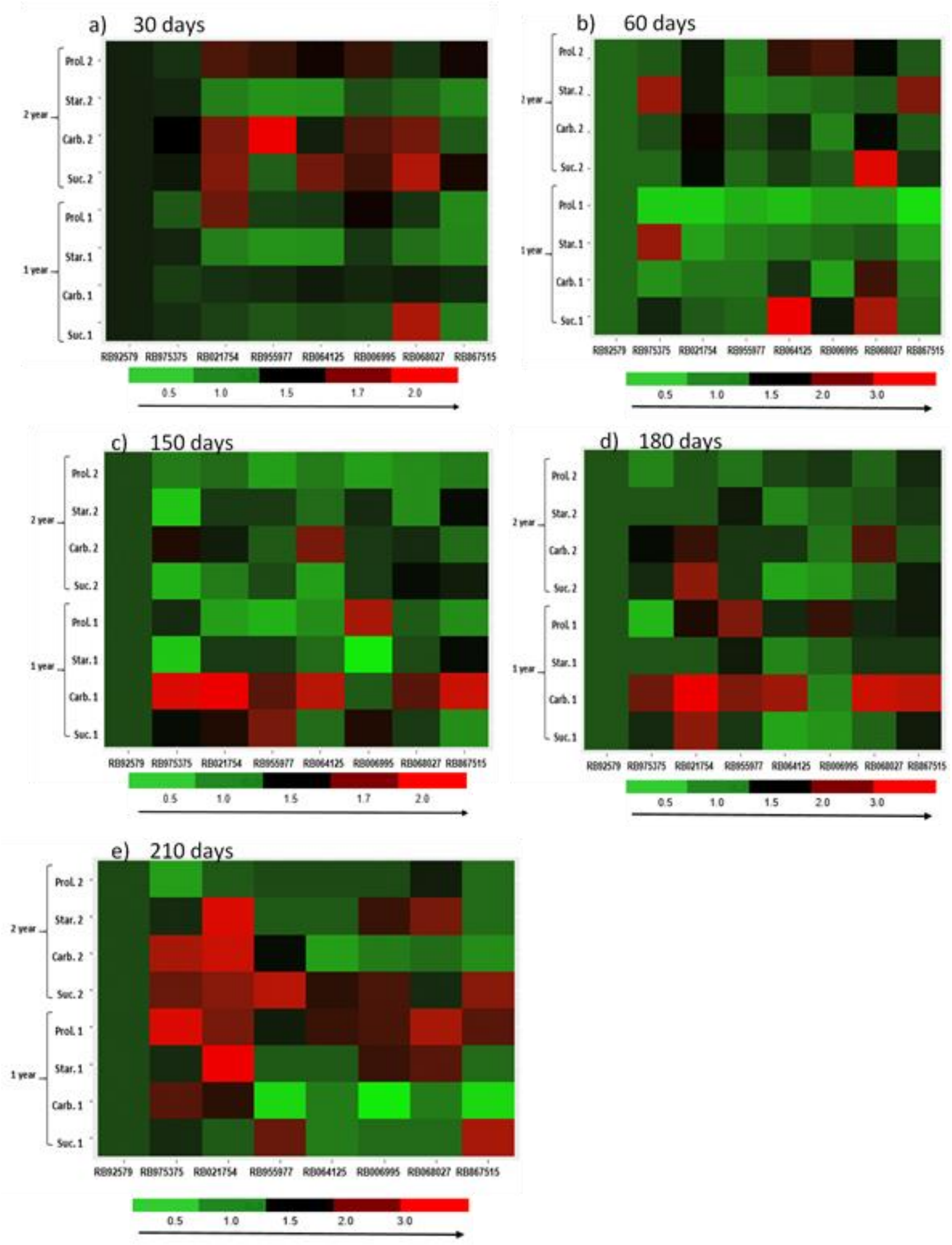
a) 30 days
b) 60 days
c) 150 days
d) 180 days
e) 210 days
2 year
1 year
Prol. 2
Star. 2
Carb. 2
Suc. 2
Prol. 1
Star. 1
Carb. 1
Suc. 1
RB92579
RB975375
RB021754
RB955977
RB064125
RB006995
RB068027
RB867515
0.5
1.0
1.5
1.7
2.0
3.0

Figure 5.

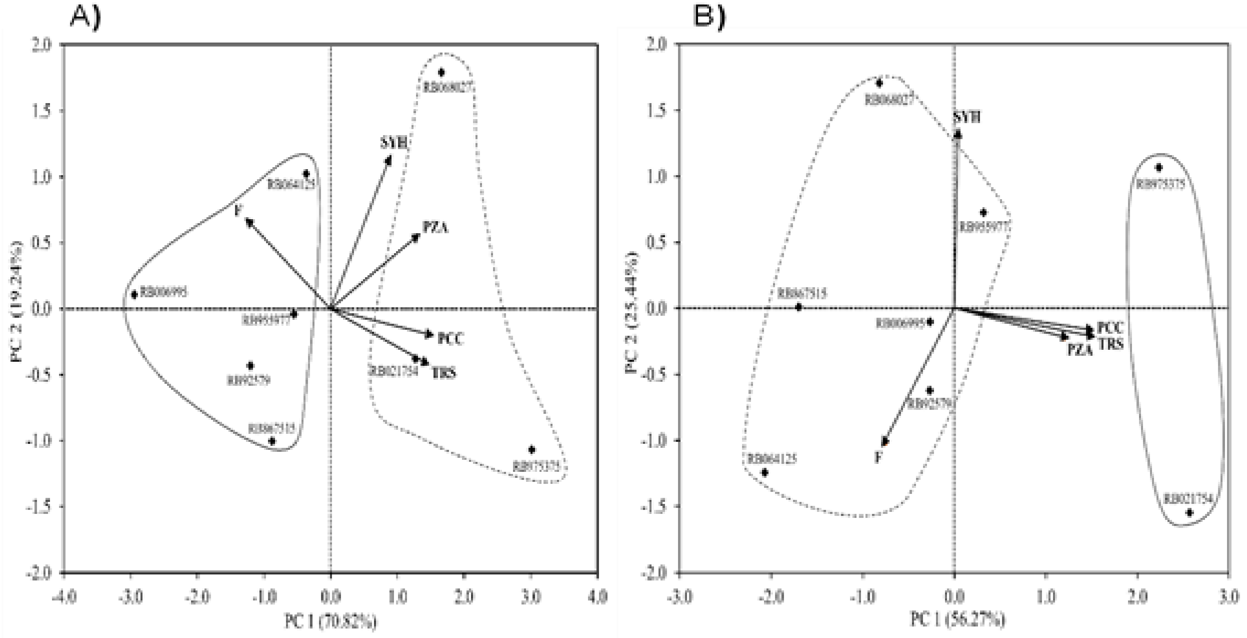
A)
PC 1 (70.82%)
PC 2 (19.24%)
RB068027
SYH
RB064125
F
PZA
RB006995
RB955977
PCC
RB92579
RB021754
TRS
RB867515
RB975375
B)
PC 1 (56.27%)
PC 2 (25.44%)
RB068027
SYH
RB975375
RB955977
RB867515
RB006995
PCC
TRS
PZA
RB92579
F
RB064125
RB021754

Figure 6.

7 - CONSIDERAÇÕES FINAIS

Um dos principais motivos que limita a expansão do setor canavieiro, no Brasil e no mundo, se deve ao fato de grande parte das áreas de cultivo da cana-de-açúcar estarem localizadas em regiões com baixas precipitações ou que apresentam regimes pluviométricos irregulares que, muitas vezes, não atendem a demanda hídrica da cultura, e influenciam o rendimento final e a qualidade tecnológica.

Este estudo, pelo fato de ser conduzido em condições de campo, retrata a realidade do cultivo em que todos os elementos climáticos atuam sobre o comportamento das plantas, onde os clones avaliados foram submetidos a um período longo e severo de seca em algumas fases do ciclo.

No primeiro capítulo avaliando, o cultivo da cana planta, observa-se que a eficiência fotossintética e o ajuste osmótico estão intimamente relacionados com a produtividade da cultura, resultando na maior tolerância às condições de limitação hídrica. As condições ambientais exibiram efeitos significativos, na maioria das características biométricas analisadas, o que pode ser indicio de boa adaptação à restrição hídrica.

Os clones que exibiram maiores crecimentos em altura e índice de área foliar, apresentaram também maior rendimento de açúcar, além de ressaltar melhores parâmetros fisiológicos, sobretudo, taxas de assimilação e transpiração de CO_2 elevadas, condutância estomática, diâmetro do caule e índice de área foliar. Situação inversa se observou nos clones considerados sensíveis à limitação hídrica imposta, onde ser verificou a redução na qualidade do produto comercial e no rendimento de açúcar.

No segundo capítulo, objetivando avaliar a variação temporal dos carboidratos e de prolina, como também as diferenças na eficiência do uso da água, nos níveis totais recuperáveis de açúcar e na produtividade da cana-de-açúcar, quando cultivada em condições de sequeiro em dois ciclos de cultivos (cana planta e primeira soca), observou-se que plantas sujeitas à limitação hídrica exibem aumento nos teores de carboidratos, em razão da redução na fotossíntese e mobilização do amido.

Outra linha de raciocínio é que o aumento no investimento inicial em área foliar, temperatura ótima de assimilação de CO_2 e diminuição da razão amido/sacarose à noite, além do favorecimento da partição de fotoassimilados para sacarose, traduz em maior crescimento das plantas. Alguns clones exibiram pouca variação no conteúdo de carboidratos, permanecendo com baixo conteúdo mesmo com limitação hídrica severa.

Os altos níveis de sacarose refletiram em elevadas médias nas avaliações tecnológicas, visto que o acúmulo de sacarose envolve o estágio principal do desenvolvimento das plantas e maturação, que são dependentes do processo fisiológico de formação de açúcares nas folhas e seu deslocamento e armazenamento no colmo.

Observou-se aumento progressivo no conteúdo de prolina com a redução do potencial de água da folha, nos dois anos de avaliação. Esse fato pode ter ajudado a manter a integridade das estruturas de cloroplastos, minimizando os danos aos mecanismos fotoquímicos do PSII causados pela restrição de água.

Fica evidenciado que os parâmetros biométricos, bioquímico-fisiológicos, tecnológicos e produtivos se associam sinergicamente, favorecendo o desenvolvimento das plantas. A diferença de precipitação ocorrida nos dois anos de avaliação pode ter proporcionado diferenças no investimento em área foliar, que se traduz em maior taxa de assimilação e, por conseguinte, ao aumento de biomassa.

Em suma, esta pesquisa possibilitou nortear novos estudos a partir dos clones promissores RB975375, RB064109 e RB068027, abordando esses parâmetros, em outros anos e locais de cultivo, como também a inclusão de outros parâmetros que permitam validar os resultados dessa investigação.