Produtividade e qualidade de grãos de arroz irrigado em safras com regimes térmicos distintos na floração

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Resumo – A temperatura é um dos fatores climáticos mais importantes para o cultivo de arroz. Temperaturas acima de 35°C limitam a produção deste cereal, podendo acarretar prejuízos irreversíveis à produtividade. A antese é a fase mais sensível do arroz à ocorrência de altas temperaturas. Este trabalho foi conduzido com o objetivo de avaliar o desempenho agronômico e a qualidade de grãos de cinco genótipos (Epagri 109, SCS116 Satoru, SCS121 CL, SCS122 Miura e SC 676), em duas safras agrícolas (2016/17 e 2017/18), correlacionando-os com a temperatura do ar durante a antese e o enchimento de grãos. Avaliaram-se a esterilidade de espiguetas, a produtividade, a massa de 1.000 grãos e aspectos qualitativos de pós-colheita (rendimento de engenho e percentual de gesso). As temperaturas observadas na safra 2016/17 da antese até a colheita foram superiores às observadas na safra 2017/18, apresentando médias de 30,5 e 28,5°C e temperaturas máximas de 34,3 e 30,9°C para cada safra, respectivamente. As temperaturas mais elevadas na primeira safra reduziram a produtividade, a massa de 1.000 grãos e o rendimento de engenho. Elas também aumentaram a esterilidade de espiguetas e o percentual de gesso. A linhagem SC676 apresentou a maior produtividade (13.070kg ha^{-1}), porém foi o genótipo com pior qualidade de grãos.

Termos para indexação: *Oryza sativa;* Calor; Esterilidade; Qualidade de grãos.

Grain yield and quality of irrigated rice in seasons with different thermal regimes at flowering

Abstract – Temperature is one of the most important climatic factors for rice production. Temperatures above 35°C limit the production of this cereal, damaging irreversibly its yield. Anthesis is the most sensitive phase of rice to the occurrence of high temperatures. This experiment was carried out aiming to evaluate the agronomic performance and grain quality of five rice genotypes (Epagri 109, SCS116 Satoru, SCS121 CL, SCS122 Miura and SC 676) at two growing seasons (2016/17 and 2017/18), correlating them with the air temperature during anthesis and grain filling. The following variables were assessed: spikelet sterility, yield, mass of 1,000 grains and postharvest qualitative aspects (milling yield and chalky grain percentage). The temperatures recorded in the 2016/17 growing season from anthesis to harvest were higher than in 2017/18. They present averages of 30.5 and 28.5°C and maximum temperatures of 34.3 and 30.9°C for each experimental year, respectively. The higher temperatures of the first growing season reduced grain yield, 1,000 grain mass and milling yield. They also increased spikelet sterility and chalky grains. The inbreed line SC 676 presented the highest yield (13,070kg ha^{-1}), however, it was also the genotype with worst grain quality.

Index terms: *Oryza sativa;* Heat; Sterility; Grain quality.

Introdução

O arroz é um cereal amplamente cultivado, podendo ser produzido em diferentes condições ambientais, sistemas de produção e regiões geográficas. Em função disto, as plantas ficam sujeitas à ocorrência de temperaturas desfavoráveis durante seu cultivo. A temperatura é um dos elementos climáticos de maior importância para a cultura. Temperaturas elevadas podem prejudicar todo o cultivo, da germinação à maturação de grãos (SOSBAI, 2016). O período reprodutivo é o mais sensível às altas temperaturas, principalmente durante a antese, onde a ocorrência de ventos quentes afeta a fecundação dos estigmas, reduzindo o número de grãos formados (CHEN et al., 2017).

A aparência dos grãos de arroz é prejudicada quando o seu desenvolvimento ocorre sob temperatura elevada, tornando-os gessados, o que afeta o polimento e leva à quebra dos grãos durante o beneficiamento (ALI et al., 2019; CHEN et al., 2017; LYMAN et al., 2013; SREENIVASULU et al., 2015; ZHAO & FITZGERALD, 2013). A formação de gesso durante o enchimento dos grãos sob temperatura alta ocorre devido ao acúmulo irregular de fotoassimilados no endosperma, o que reduz a resistência do grão às forças aplicadas durante o beneficiamento, predispondo a uma quebra quando os grãos estão sendo polidos, afetando diretamente o rendimento de engenho (LONDERO et al., 2015).

Diante da instabilidade térmica e da constância de mudanças climáticas nos últimos anos, a ocorrência de altas

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temperaturas durante o ciclo de cultura do arroz é comum no sul do Brasil, que abrange os dois maiores produtores nacionais deste cereal, Rio Grande do Sul e Santa Catarina (SOSBAI, 2016). Apesar da evolução da produtividade, observa-se uma redução nos anos em que ocorrem temperaturas altas na fase reprodutiva da cultura, o que afeta também a qualidade de grãos. Registros de redução da produtividade devido ao estresse por altas temperaturas foram identificados em diversas safras em Santa Catarina. Um exemplo disto ocorreu na safra 2009/10, na qual temperaturas entre 33 e 40°C foram registradas, por períodos de três a sete dias, durante os meses de janeiro a março. Este estresse diminuiu a produtividade e a qualidade de grãos nas principais regiões produtoras de arroz do Estado (EBERHARDT, 2010).

Este trabalho foi conduzido com objetivo de avaliar a produtividade e a qualidade dos grãos de genótipos de arroz irrigado em duas safras agrícolas com diferentes regimes térmicos.

Material e métodos

O experimento foi conduzido no município de Itajaí, SC, durante as safras de 2016/17 e 2017/18. O delineamento experimental foi inteiramente casualizado com três repetições e esquema bifatorial (5x2), sendo o primeiro fator os genótipos utilizados (as cultivares SCS122 Miura, SCS121 CL, Epagri 109 e SCS116 Satoru e a linhagem SC 676) e o segundo fator as safras com diferentes regimes térmicos. Os genótipos são pertencentes à subespécie índica e apresentam ciclo tardio.

Os experimentos foram conduzidos no sistema pré-germinado de cultivo. As semeaduras foram realizadas em 29/09/2016 e 26/10/2017, com densidade de 120kg ha^{-1} de sementes nos dois anos. As parcelas tinham 2 x 5m de dimensão total e uma área útil de 1,5 x 4m.

O manejo da cultura nos experimentos seguiu as recomendações da Sosbai (2016), com aplicações de uma dose de P_2O_5 na base e três doses de N+K_2O na cobertura, nos estádios V2, V4 e V8 (COUNCE et al., 2000). Durante o cultivo foram realizadas pulverizações de fungicidas nas combinações de Tebuconazol + Trifloxystrobina e Triazol + Estrobirulina, o que propiciou ausência de doenças durante o cultivo das plantas de arroz deste experimento.

A antese foi determinada quando 50% das panículas de cada genótipo, em cada safra, estavam no estádio R4 (COUNCE et al., 2000), ocorrendo de 17/01/2017 a 29/01/2017 e 05/02/2018 a 23/02/2018, na 1ª e 2ª safra, respectivamente. As colheitas ocorreram em 13/03/2017 e 22/03/2018. Elas foram efetuadas com colheitadeira de parcelas automatizada Classic/Weitersteiger numa área útil de 6m^2 para determinação da produtividade, que foi expressa em kg ha^{-1}, na umidade de 13%.

Para determinação da esterilidade foram colhidas amostras de 1m^2 em cada parcela, que foram secadas e debulhadas manualmente. Em seguida, separaram-se as espiguetas cheias e estéreis, que foram contadas no contador de grãos Sanick ESC 2008. Posteriormente, determinou-se o percentual de esterilidade de espiguetas das parcelas. As espiguetas cheias foram utilizadas para determinação da massa de 1.000 grãos que também foi expressa na umidade de 13%.

Para avaliação dos aspectos de pós-colheita (rendimento industrial e qualidade de grãos) foram pesadas 50g de cada parcela. Estas amostras foram processadas no engenho de prova – Suzuki, para obtenção de arroz descascado e polido. Em seguida, as amostras foram submetidas à análise no scanner de grãos Image – Rice Grain Scanner (Selgron), que está ligado a um computador acoplado a um sistema digital de captura de imagens e um software de análise de grãos. As variáveis utilizadas foram o rendimento de engenho, o percentual de grãos gessados e o percentual de área gessada dos grãos. A determinação do percentual de grãos gessados levou em consideração o percentual de grãos que apresentavam centro branco, independentemente do tamanho. A área gessada dos grãos foi determinada considerando o percentual total da área gessada na amostra.

Os dados obtidos foram avaliados estatisticamente através da análise de variância, utilizando o programa Assistat Software Version 7.7 (SILVA & AZEVEDO, 2016). Os valores de F foram considerados ao nível de significância de 5% ($P<0,05$). Quando significativas, as médias foram comparadas pelo teste de Tukey, também ao nível de 5% de probabilidade de erro.

Resultados e discussão

Houve diferenças climáticas importantes entre as safras quanto à temperatura e radiação solar (Tabela 1). Na safra 2016/17, a temperatura média do cultivo, entre semeadura e colheita, foi de 28,1°C. Do florescimento (R4) até a maturação de colheita (R9), a temperatura média foi de 30,5°C. Considerando apenas o florescimento, a temperatura média foi 30,6°C, com máxima observada de 34,3°C. Na safra seguinte (2017/18), a temperatura média durante o ciclo total também foi de 28,1°C. Contudo, do florescimento (R4) até a maturação de colheita (R9) a temperatura média foi de 28,5°C. Considerando apenas o florescimento, a temperatura média foi menor (28,2°C), com temperatura máxima de 30,9°C (Tabela 1). A safra 2016/17 registrou a maior disponibilidade de radiação, em todas as fases do ciclo da cultura, o que contribuiu para os maiores valores de temperatura na mesma (INMET, 2019).

A análise de variância mostrou que o efeito do fator genótipo foi significativo para produtividade (Tabela 3) e do fator safra para produtividade, esterilidade e rendimento de engenho (Tabela 2). Houve interação significativa entre safra e genótipo somente para as variáveis massa de 1.000 grãos e gesso dos grãos (Tabela 3).

Houve um aumento de 3,7% na esterilidade de espiguetas da safra 2017/18 para a 2016/17 (Tabela 2), quando as temperaturas mais elevadas foram registradas durante a antese. Temperaturas acima de 32,6°C durante a floração podem causar até 50% de esterilidade (NGUYEN et al., 2014), enquanto valores acima de 35°C causam até 100% de esterilidade, dependendo do genótipo (VAN OORT et al., 2014). Altas temperaturas na antese promovem a redução ou o impedimento da deiscência de anteras, da liberação de grãos de pólen e da▸

Tabela 1. Temperatura e radiação solar em diferentes fases do ciclo do arroz, nas safras de 2016/17 e 2017/18
Table 1. Temperature and solar radiation at different stages of the rice cycle, in the 2016/17 and 2017/18 growing seasons

Fase	Temperatura média (°C)		Radiação solar média (kJ m^{-2})	
	2016/17	2017/18	2016/17	2017/18
Ciclo total	28,1	28,1	4.770,1	4.373,2
Antese (R4)	30,6	28,2	4.743,9	4.527,6
Máxima na antese (R4)	34,3	30,9	-	-
Antese à maturação (R4-R9)	30,5	28,5	4.210,3	3.693,6

Tabela 2. Esterilidade de espiguetas (%), produtividade (kg ha^{-1}) e rendimento de engenho (%) em safras com temperaturas distintas, na média de cinco genótipos
Table 2. Spikelet sterility (%), productivity (kg ha^{-1}) and milling yield (%) in growing seasons with different temperatures, averaging five genotypes

	2016/17	2017/18	
Esterilidade de espiguetas (%)	17,3 A*	13,6 B	CV% = 21,92
Produtividade (kg ha^{-1})	11.123 B*	12.714 A	CV% = 7,27
Rendimento de engenho (%)	64,8 B*	69,3 A	CV% = 4,74

*Médias seguidas de mesma letra maiúscula na linha não diferem pelo teste de Tukey (p ≤0,05).

Tabela 3. Produtividade (kg ha^{-1}) dos genótipos avaliados, na média de duas safras, e massa de 1.000 grãos (g), grãos gessados (%) e área gessada (%) nas duas safras com regimes térmicos distintos
Table 3. Yield (kg ha^{-1}) of the evaluated genotypes, averaging two harvests, and a mass of 1,000 grains (g), chalky grains (%) and chalky area (%) in the two growing seasons with different thermal regimes

	Produtividade (kg ha^{-1})	Massa de 1.000 grãos (g)		Grãos gessados (%)		Área gessada (%)	
		2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
SC 676	13.070 a*	27,9 Bab*	30,1 Aa	17,4 Aa*	5,1 Ba	13,8 Aa*	2,7 Ba
SCS122 Miura	12.298 ab	26,9 Bb	30,0 Aa	7,3 Ab	3,2 Bab	4,6 Ab	1,8 Bab
SCS116 Satoru	11.962 abc	28,3 Ba	30,1 Aa	6,4 Abc	2,8 Bab	3,3 Abc	1,5 Bab
Epagri 109	11.501 bc	28,9 Aa	29,6 Aa	6,0 Abc	3,1 Bab	3,9 Abc	1,9 Bab
SCS121 CL	10.760 c	28,0 Bab	30,4 Aa	4,4 Ac	1,5 Bb	2,1 Ac	0,6 Bb
	CV% = 7,27	CV% = 1,75		CV% = 20,10		CV% = 22,05	

*Médias seguidas de mesma letra, maiúscula na linha e minúscula na coluna, não diferem teste de Tukey (p ≤ 0,05).

elongação do tubo polínico, causando a esterilidade de espiguetas, que também depende do tempo de exposição ao estresse térmico, havendo maior índice de esterilidade no período de seis horas do que de duas horas sob estresse a 38°C (JAGADISH et al., 2008).

A massa de 1.000 grãos de todos os genótipos, com exceção do cultivar Epagri 109, foi maior na safra 2017/18 do que na safra 2016/17 (Tabela 3). Temperaturas elevadas após a floração aumentam a atividade respiratória da planta e aceleram o enchimento de grãos, diminuindo a duração deste período (SOSBAI, 2016). Estes dois fatores provavelmente contribuíram para a redução na massa de 1.000 grãos de quatro dos cinco genótipos avaliados na primeira safra.

Observando-se os valores da massa de 1.000 grãos em cada safra, verificou-se que na safra 2017/18 não houve diferença entre os genótipos para esta variável. Isto sugere que temperaturas favoráveis ao cultivo de arroz após a floração não afetam a massa de 1.000

grãos dos genótipos avaliados. Em contrapartida, ao avaliar esta característica na safra 2016/17, cujas temperaturas foram mais elevadas durante o florescimento e o enchimento de grãos, houve diferença significativa entre os genótipos quanto à massa de 1.000 grãos. Nesta safra, os genótipos que se destacaram foram os cultivares Epagri 109 e SCS116 Satoru (Tabela 3), que diferiram significativamente do cultivar SCS122 Miura, que apresentou a menor massa de 1.000 grãos. Os valores de referência para massa de 1.000 grãos dos cultivares avaliados neste estudo são de 28,5, 30,5, 29,9 e 28,6g, para Epagri 109, SCS116 Satoru, SCS121 CL e SCS122 Miura, respectivamente (EPAGRI, 2019). Deste modo, verificou-se que o cultivar Epagri 109 apresentou, em ambas as safras, massa de 1.000 grãos superior ao valor de referência. O contrário foi observado no cultivar SCS116 Satoru, que apresentou valores menores que o de referência, independentemente da safra analisada. Os cultivares SCS121 CL e SCS122 Miura apresentaram o mesmo comportamento, tendo obtido menores valores da massa de 1.000 grãos na safra 2016/17 e maiores na safra 2017/18, comparado aos seus valores de referência (valores apresentados durante o lançamento dos cultivares).

A produtividade dos genótipos avaliados oscilou entre 10.760 e 13.070kg ha^{-1} (Tabela 3). A linhagem SC 676 foi a mais produtiva, no entanto não diferiu significativamente dos cultivares SCS122 Miura e SCS116 Satoru. Comportamento semelhante foi observado em trabalhos conduzidos sob baixas temperaturas por Marschalek et al. (2015; 2019), Souza (2015) e Souza et al. (2017). As menores produtividades foram registradas nos cultivares Epagri 109 e SCS121 CL. Todos os cultivares apresentaram produtividades acima dos valores de referência em seus lançamentos, que foram de 9.500, 9.400, 10.200 e 10.400kg ha^{-1}, para SCS122 Miura, SCS116 Satoru, Epagri 109 e SCS121 CL, respectivamente (EPAGRI, 2019).

A produtividade da segunda safra foi 14% superior à da primeira, na média dos cinco genótipos (Tabela 2). As temperaturas mais altas registradas durante a antese e o enchimento de grãos em 2016/17 aumentaram a esterilidade de espiguetas (Tabela 2) e reduziram a massa de 1.000 grãos (Tabela 3), como descrito anteriormente, contribuindo para que a produtividade fosse menor em 2016/17 do que em 2017/18.

O percentual de grãos gessados e a área gessada dos grãos apresentaram mesma tendência de resposta. Os maiores valores de gesso foram observados na safra 2016/17, onde ocorreram as maiores temperaturas da antese à colheita (Tabela 3). Isto comprova que a ocorrência de temperaturas altas durante o período de enchimento de grãos aumenta a incidência de grãos gessados, independentemente do parâmetro utilizado para avaliá-lo.

A percentagem de grãos gessados para os genótipos lançados pela Epagri deve ser menor que 5% (EPAGRI, 2019). Os valores registrados no trabalho variaram de 1,5 a 17,4%, dependendo do genótipo e da safra (Tabela 3). Os maiores percentuais foram da linhagem SC 676, em ambas as safras. Os menores valores foram apresentados pelo cultivar SCS121 CL. Os cultivares SCS122 Miura, SCS116 Satoru e Epagri 109 apresentaram valores intermediários e similares em relação ao percentual de grãos gessados, com média de 6,6% na primeira safra e 3,0% na segunda. A maior variação observada entre safras foi na linhagem SC 676, onde o percentual de grãos gessados reduziu 3,4 vezes na safra 2017/18, em relação à safra 2016/17. Isto indica que o decréscimo das temperaturas durante o enchimento de grãos foi mais benéfico a este genótipo.

Para área gessada dos grãos, os dados obtidos variaram de 0,6 a 13,8%, em função da safra e do genótipo avaliado (Tabela 3). A linhagem SC 676 apresentou maior percentual de área gessada nas duas safras. Os menores percentuais foram registrados no cultivar SCS121 CL. Os demais cultivares avaliados apresentaram valor intermediário de área gessada, com médias de 3,9 e 1,7%, para as safras 2016/17 e 2017/18, respectivamente. A maior diferença entre safras ocorreu na linhagem SC 676, onde houve redução de 5,1 vezes de área gessada dos grãos na segunda safra em relação à primeira.

O rendimento de engenho (percentual de grãos beneficiados: inteiros e quebrados) dos genótipos desenvolvidos pela Epagri deve apresentar valores acima de 60%. Quanto maior esse valor, melhor é a qualidade do produto beneficiado. Os cultivares da Epagri, SCS122 Miura, SCS121 CL, SCS116 Satoru e Epagri 109, apresentam valores referência de 67,5, 71,0, 70,0 e 71,0%, em relação ao rendimento de engenho (EPAGRI, 2019). Nas duas safras o rendimento médio de engenho dos cinco genótipos foi inferior a 70% (Tabela 2).

O rendimento de engenho foi menor na safra 2016/17 do que na safra seguinte (Tabela 2). Na primeira safra foram registrados os maiores valores de temperatura durante o período de enchimento de grãos. Este resultado vai ao encontro das observações feitas por Shrivastava et al. (2012) e Sreenivasulu et al. (2015), que observaram que o rendimento de engenho em arroz diminui 6% a cada 1°C de aumento na temperatura do ar.

Durante o desenvolvimento dos grãos de arroz, temperaturas elevadas comprometem visual e estruturalmente os grãos. O calor favorece a formação de gesso devido ao acúmulo irregular e pouco compactado dos fotoassimilados no endosperma. Consequentemente, os grãos ficam mais frágeis durante o processamento industrial, aumentando a quebra e diminuindo o rendimento de engenho (ALI et al., 2019; LYMAN et al., 2013; LONDERO et al., 2015; SREENIVASULU et al., 2015; ZHAO & FITZGERALD, 2013).

De modo geral, os resultados obtidos comprovam as observações feitas por Beckles & Thitisaksakul (2013) e Londero et al. (2015), de que a aparência do grão de arroz é prejudicada pela elevação da temperatura durante o desenvolvimento do grão. Segundo Lyman et al. (2013), o aumento da temperatura durante o enchimento de grãos fomenta a quebra e o gesso dos grãos e reduz a produtividade. Estes autores constataram que o aumento de 1, 2 e 4°C na temperatura reduziu a produtividade em até 6,2, 13,5 e 30,0%, respectivamente.

A temperatura média durante todo o cultivo, em ambas as safras, foi a mesma (28,1°C). Já a diferença observada nas temperaturas durante o período antese-maturação foi de 2°C (Tabela 1). Isto ressalta que a diferença obtida no ▸

percentual de esterilidade, na produtividade e na qualidade de grãos entre as safras deveu-se, principalmente, à ocorrência de temperaturas superiores na primeira safra, tanto na floração quanto durante o enchimento de grãos.

Os dados relacionados a produtividade e qualidade de grãos demonstram resultado inversamente proporcional à esterilidade. Assim, pode-se inferir que o aumento de esterilidade reduz a produtividade e a qualidade de grãos, o que também foi observado por Beckles & Thitisaksakul (2013), Eberhardt (2010), Shrivastava et al. (2012) e Sreenivasulu et al. (2015).

Conclusões

A produtividade e a qualidade de grãos dos genótipos avaliados diminuíram na safra que apresentou maior temperatura média e máxima na antese e na fase de enchimento de grãos.

A linhagem SC 676 destacou-se como o genótipo mais produtivo e o que apresentou maior aumento no gesso na safra com temperatura média mais alta.

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Effects of Single and Split Pre-Harvest Aminoethoxyvinylglycine Applications in Bioactive Compounds and Antioxidant Activity in 'Baigent' Apples Under Anti-Hail Nets

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Abstract

The objective of this study was to evaluate the effects of single versus split pre-harvest applications of aminoethoxyvinylglycine (AVG) on the concentrations of bioactive compounds and antioxidant activity in 'Baigent' apple fruit cultivated under anti-hail nets, assessed at harvest and after cold storage. The pre-harvest application of AVG in a single dose (125 mg L^{-1}) in 'Baigent' apple trees reduces the total antioxidant activity and concentration of anthocyanins and the total phenolic compound and chlorogenic acid in the fruit skin, both at harvest and after cold storage and reduces, in the skin, the concentrations of epicatechin at harvest and of quercetin after the cold storage. The parceled application of AVG (62.5 mg L^{-1} + 62.5 mg L^{-1}) does not influence or present a less-pronounced negative effect on the total antioxidant activity and the contents of the total phenolic compound and anthocyanins in the fruit skin. Split AVG application can help maintain fruit quality during storage, providing a practical strategy for producers to optimize both marketable quality and nutritional value, potentially positively impacting commercial returns.

Keywords: *Malus domestica*; shading; growth regulators; cold storage; polyphenols; anthocyanins

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1. Introduction

The aminoethoxyvinylglycine (AVG) compound is a growth regulator applied to the field intending to delay the harvest and reduce fruit fall, in addition to delaying ripening and improving quality maintenance during storage due to reduced ethylene production [1,2]. In the ethylene biosynthesis route, AVG inhibits the conversion of S-adenosylmethionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC), cat-alyzed by the enzyme ACC synthase (ACS) [3]. Potential risks associated with this substance revolve mainly around residual toxicity and possible impacts on human health and the environment. According to leading regulatory agencies, such as the United States Environmental Protection Agency (EPA) and European agencies, AVG is considered safe for agricultural use when applied strictly according to manufacturer instructions and in line with national and international regulations. Due to its low tox-icity, risks to human health are minimal, especially when pre-harvest intervals and recommended doses are observed.

AVG residues in food remain well below established maximum residue limits, posing no risk to consumers. However, misuse—such as ex-ceeding recommended doses or applying outside of the specified period—could pose environmental and health risks, highlighting the importance of applicator training and strict adherence to official guidelines.

The application of AVG at the recommended dose and time [125 mg L^{-1}, applied 30 days before the estimated commercial harvest date (DBEHD)], causes a reduction in the red coloration in apple skin [4,5]. It was demonstrated in 'Honeycrisp' and 'Gala' ap-ples that, despite delaying fruit ripening, AVG had a negative impact on the develop-ment of the red color [1,6]. For commercial purposes, at least 50% of the apple skin must be covered with red pigmentation to meet market acceptance standards [6]. Furthermore, AVG can alter the constitution of bioactive compounds, as well as the antioxidant potential of fruits [7].

The apple (Malus domestica) represents an important source of vitamin C, flavo-noids, and phenolic compounds [8]. These compounds present benefits to human health through the prevention of several diseases [9] due to their antioxidant potential [10]; compounds are the most important contributors to total antioxidant activity in apples [11,12] and are derived from secondary metabolism in plants, which perform essential functions in reproduction, growth, and defense mechanisms, as well as contribute to the coloring of plants, flowers, and fruits [13,14]. They have high antioxidant potential due to their ability to neutralize and sequester free radicals [10].

The main groups of phenolic compounds in apples are phenolic acids, dihydrochalcones, flavonols, flavanols (flavan-3-ols), and anthocyanin [15,16]. According to Jakobek et al. [16] and Ceymann et al. [15], phenolic acids, dihydrochalcones, and flavonols contribute, respectively, 3–30%, 1–5%, and 2–10% of the total phenolic compound content in apples. Flavan-3-ols in monomeric forms [(+)-catechin and (−)-epicatechin] and oligomeric (proanthocyanidins) are the main flavanols and contribute 55–85% of the total phenolic compound content in apples. Anthocyanins are present in red or partially red apple cultivars, and their contribution varies from 1 to 7% of the phenolic compound content. The occurrence and content of these compounds vary between cultivars [11,16,17], skin and flesh tissue [17], orchard management [18], and storage conditions [17,19].

Currently, most apple orchards in southern Brazil are cultivated under anti-hail nets, which reduce solar radiation incident on the plants and, therefore, can interfere with the quality of the fruits [20,21]. The parceled application of the recommended dose of AVG (62.5 mg L^{-1} + 62.5 mg L^{-1}, applied at 20 and 30 DBEHD) has shown posi-tive results in the delay of ripening and maintenance of the quality of stored fruits, without causing damage to the formation of the red color of apples [17,22]. Preharvest application of aminoethoxyvinylglycine (AVG) was more effective than 1-methylcyclopropene (1-MCP) in maintaining suppressed ethylene production in 'NY2' apples (RubyFrost®) during extended storage periods [23]. However, studies of the effect of AVG on anthocyanin, total phenolic compound, and total antioxidant ac-tivity content in apples grown under anti-hail nets are limited. Also, no information was found on the effect of AVG applied at the recommended dose and time (125 mg L^{-1} applied at 30 DBEHD), as well as on its split (62.5 mg L^{-1} + 62.5 mg L^{-1}, applied 20 and 30 DBEHD), on the functional properties of 'Baigent' apples grown under anti-hail nets, at harvest and after cold storage.

The objective of this study was to evaluate the effects of single versus split pre-harvest applications of aminoethoxyvinylglycine (AVG) on the concentrations of bioactive compounds and antioxidant activity in 'Baigent' apple fruit cultivated under anti-hail nets, assessed at harvest and after cold storage.

2. Materials and Methods

2.1. Location of the Orchard

The experiment was carried out from January to December 2018 with 'Baigent' apples in a commercial orchard located in the municipality of Vacaria, RS, Brazil (50°42′ W; 28°33′ S; and 955 m above sea level), covered with black anti-hail nets with a mesh opening of 4 × 7 mm, 25% to 35% photosynthetic active radiation (PAR), installed in 2010. The orchard was composed of 7-year-old trees, grafted on M9 rootstock, with spacings of 3.5 m × 0.45 m. The soil of the experimental field is a Latosol Bruno Aluminum—LBa, according to the Brazilian soil classification system [24]. According to the Köppen–Geiger's classification, the climate is 'Cfb', constantly moist temperate with mild summers.

2.2. Treatments

The treatments consisted of a control (plants sprayed with water); single-dose AVG (125 mg L^{-1}, sprayed 30 DBEHD); and split-dose AVG (62.5 mg L^{-1} + 62.5 mg L^{-1}, sprayed 30 and 20 DBEHD). The source of AVG was ReTain®. The adhesive spreader used in the treatments was Break Thru (0.05% v/v). For each treatment, two harvests were performed: the first at the commercial ripening stage of the control treatment and the second 14 days later. The fruit (100) were randomly harvested from the middle third of the plant canopy. For storage, 20 fruits per experimental unit were used, without damage or defects. The choice of 20 apples per batch is standard in postharvest studies to ensure statistical power and reduce variability; this batch size is commonly supported in the literature. The applied AVG dosages (single: 125 mg L^{-1}; split: 62.5 mg L^{-1} + 62.5 mg L^{-1}) follow agronomic recommendations and previously published studies.

2.3. Storage Conditions and Analyzed Variables

The fruits were evaluated at harvest and after four months of cold storage (0.5 ± 0.2 °C and RH 92 ± 5%), followed by seven days in ambient conditions (20 ± 5 °C and RH 63 ± 2%) to simulate the marketing period. The following attributes were evaluated: total phenolic compound (mg GAE kg^{-1} FW) and total antioxidant activity (DPPH and ABTS methods; µMol trolox kg^{-1} FW) in the skin and flesh, and total anthocyanin (mg cyani-dine 3-glucoside kg^{-1} FW) and chlorogenic acid, floridizine, epicatechin, and procyanidin B1 (mg kg^{-1} FW in the skin). The evaluations were analyzed as described in Stanger et al. [17]. The analysis of chlorogenic acid, floridizine, epicatechin, and procyanidin B1 were only performed in Harvest 2.

Analytical grade reagents, including 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu rea-gent, sodium acetate, and potassium persulfate, were sourced from Sigma Chemical Co. (St. Louis, MO, USA). Standard solutions for chlorogenic acid, catechin, epicatechin, procyanidins B1 and B2, quercetin 3-galactoside, and phloridzin, as well as HPLC-grade solvents—acetonitrile, acetic acid, and methanol—were also obtained from Sigma Chemical Co. Additional chemicals such as gallic acid, sodium carbonate, acetone, and ethanol were purchased from Vetec (Rio de Janeiro, Brazil) with analytical grade quality. Peels were carefully removed from the entire fruit surface using a sharp blade ap-proximately 1 mm thick. Pulp samples were collected by slicing a longitudinal segment (about 1 cm thick) from the fruit's center, excluding the endocarp and preserving the sides of the slice. Pulp was homogenized using a vertical grinder (model RI1364, Philips Walita, Varginha, Brazil), and peels were pulverized in a mortar with liquid nitrogen.

Extraction for total phenolic content (TPC) and total antioxidant activity (TAA) quantification followed the approach outlined by Rufino et al. [25], with modifications based on

Larrauri et al. [26] For this, 10.0 g of pulp and 2.5 g of peel were each mixed in a Falcon tube (Zollstr, Switzerland) with 10 mL of a 1:1 methanol/distilled water solution. These were homogenized using an Ultra-Turrax disperser (model D-91126, Heidolph, Schwabach, Germany) and allowed to stand for 60 min at room temperature. Fol-lowing this, samples were centrifuged in an Eppendorf 5810R centrifuge (Hamburg, Germany) at 12,880 g for 20 min at 4 °C. The supernatant was decanted into a 25 mL volumetric flask, and a second extraction was performed by treating the residue with 10 mL of 70:30 acetone/distilled water. The mixture was homogenized and left to stand for another 60 min at 20 °C before another round of centrifugation under the same conditions. The second supernatant was combined with the first, and the volume was topped up to 25 mL with distilled water.

To determine TPC, a modified version of the spectrophotometric Folin–Ciocalteu method, described by Roesler et al. [27], was used. Each assay included 2.5 mL of a Folin–Ciocalteu/distilled water mixture (25:75, v/v) added to 0.5 mL of appropriately diluted hydroalcoholic extract in triplicate. The mixture was vortexed and allowed to react for 3 min at 20 °C. Then, 2.0 mL of sodium carbonate solution (10% w/v) was added, followed by another mixing. Samples were left in the dark for one hour before meas-uring absorbance at 765 nm using a BEL2000-UV spectrophotometer (Bel Photonics, Piracicaba, Brazil). TPC was quantified via the calibration curve with gallic acid, and results were presented as mg gallic acid equivalents per 100 g of fresh mass (mg GAE 100 g^{-1} FM).

TAA was evaluated using both ABTS and DPPH assays. For the ABTS method, radical cations were produced by incubating an ABTS stock solution (7 mM) with po-tassium persulfate (140 mM) in the dark for 16 h at 20 °C. Prior to analysis, radicals were adjusted with ethanol to reach an absorbance of 0.70 ± 0.05 at 734 nm. Three dilu-tions of extract were tested in triplicate by adding 30 µL of extract to 3.0 mL of ABTS solution, followed by vortexing; absorbance was read at 734 nm after 6 min. Trolox standards were used for calibration, and results were reported as a Trolox equivalent antioxidant capacity (TEAC) in µmol TEAC g^{-1} FM.

The DPPH test involved preparing a 0.06 mM DPPH solution in methanol on the day of testing. Then, 0.1 mL of hydroalcoholic extract was mixed with 3.9 mL of DPPH reagent in triplicate, followed by vortexing. Absorbance was measured at 515 nm after 30 min. Trolox standards generated the calibration curve, and antioxidant capacity was expressed as µmol TEAC per 100 g of fresh mass.

Total anthocyanins (TANs) were quantified using a method adapted from Fuleki and Francis [28]. For this, 5.0 g of apple peel was mixed with 15 mL of 95:5 (v/v) ethanol/distilled water and acidified with ethanol/hydrochloric acid (1.5 N HCl, 85:15, and v/v). Samples were homogenized (SilentCrusher M, Heidolph), maintained at 4 °C for 24 h, and centrifuged at 12,880 g for 20 min at 4 °C (Eppendorf 5810R). From the supernatant, 2 mL was transferred to a 50 mL volumetric flask, filled to volume with extraction solvent, and absorbance was measured at 535 nm with a BEL2000-UV spec-trophotometer. Results were expressed as mg cyanidin 3-glucoside per 100 g of fresh mass.

Phenolic compound profiling (IPC) was conducted via HPLC, following Tsao et al. [29]. Sample preparation paralleled the procedures for TPC and TAA: extracted samples (1:1 w/v) in methanol/ultrapure water (70:30, v/v) were homogenized, vacuum filtered, and further filtered through 0.45 µm syringe filters (Kasvi, Curitiba, Brazil). Samples were stored at −20 °C until analysis. HPLC analysis was performed using a Shimadzu system (Tokyo, Japan) equipped with an SCL-10AVP controller, FCV-10AL VP gradient mixer, LC-10ADVP pump, SIL 10-ADVP auto-injector, and SPD-10A VP UV detector, utilizing a CLASS VP (version 6.14) software and a C18 column (250 × 4.6 mm, 5 µm, Restek, PA, USA). The mobile phase used was acetic acid/ultrapure water (6:94, v/v) with 2 mM sodium acetate buffer, pH 2.55 (solvent A), and acetonitrile (solvent B), with a stepwise gradient elution

over 80 min at a flow rate of 1.0 mL/min. Detection occurred at 280 nm and injections were 20 µL. Phenolic compounds were identified by matching retention times with standards for procyanidins B1 (10.9 min), catechin (17.4 min), procyanidin B2 (22.0 min), chlorogenic acid (22.7 min), epicatechin (35.2 min), quercetin 3-galactoside (52.6 min), and phloridzin (67.0 min). A spectral library, in-cluding retention times and UV spectra at 280 nm, was developed for compound con-firmation. Quantification used external calibration curves (0–100 mg g^{-1}) with standards in methanol, and standard solutions were run at both the start and end of each batch to ensure accuracy, with recovery rates between 97% and 110%. All analyses were per-formed in duplicate.

2.4. Experimental Design and Statistical Analysis

The experimental design used was in random blocks, according to a 3 × 2 factorial (three treatments and two harvests) with four replicates. Each repetition is composed of 20 fruits. The data were submitted to an analysis of variance and means of treatment compared by the Tukey test ($p < 0.05$). The total phenolic compound variable was sub-mitted to a Pearson's correlation analysis ($p < 0.001$) with total antioxidant activity. The statistical analyses were performed using the SAS program (SAS Institute, 2002, version 9.0).

3. Results and Discussion

3.1. When Harvesting Fruit

A positive and highly significant correlation ($p < 0.001$) was observed between total phenolic compound content and total antioxidant activity (by the ABTS and DPPH methods) in the skin and flesh and between the total phenolic compound content and the anthocyanin content in the skin in both harvests (Table 1). Stanger et al. [17,19] and Soethe et al. [30] also observed a positive linear correlation between the total phenolic compound and total antioxidant activity in apples at harvest and after storage. This indicates that phenolic compounds are important phytochemicals that contribute to antioxidant activity in apples. The structure, particularly the number and position of the hydroxyl groups and the nature of the replacements of the aromatic rings, are the main characteristics of the phenolic compounds responsible for the antioxidant activity [31,32].

Table 1. Pearson correlation coefficients (r) between total phenolic compounds (TPC) and total antioxidant activity, quantified by ABTS and DPPH methods, and anthocyanins (ANT), in the 'Baigent' apple skin and flesh portions, evaluated in the commercial harvest, at two harvest dates.

Correlation	Harvest 1		Harvest 2	
	Skin	Flesh	Skin	Flesh
TPC × DPPH	0.99 ***	0.99 ***	0.99 ***	0.95 ***
TPC × ABTS	0.99 ***	0.81 ***	0.82 ***	0.96 ***
TPC × ANT	0.99 ***	-	0.90 ***	-

*** significant ($p < 0.001$).

No difference was observed between the treatments for the total phenolic compound and total antioxidant activity content of the flesh in the two harvests evaluated (Figure 1). Ozkan et al. [8], working with 'Braeburn' apples, and Awad and Jager [32], working with 'Jonagold' apples, also did not notice any change in the total phenolic compound content of the flesh with a pre-harvest application of AVG.

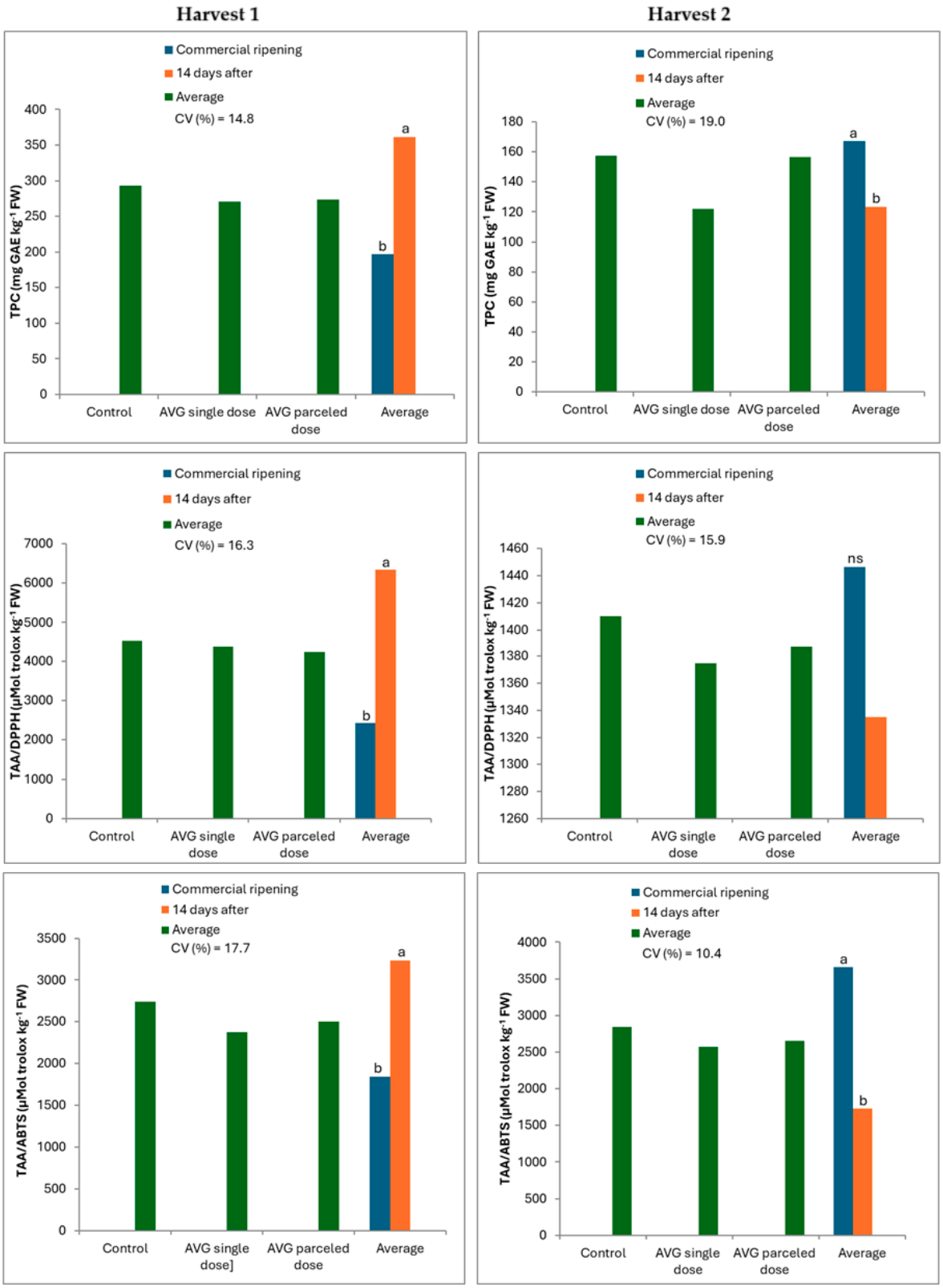

Figure 1. Total phenolic compound contents (TPC; mg GAE kg^{-1} FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and µMol trolox kg^{-1} FW) in 'Baigent' apple flesh, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—days before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates. Treatment averages, comparing harvest dates, not followed by the same lower-case letter, differ by Tukey test ($p < 0.05$). ns: non-significant difference between treatments for average data of the two harvest dates.

There was no significant interaction between treatments and harvest dates for the total phenolic compound, total antioxidant activity (ABTS and DPPH methods), and anthocyanin quantified in the fruit skin (Figure 2). Pre-harvest application of AVG in a single dose (125 mg L^{-1}) provided fruits with a lower total phenolic compound content in the skin compared to the control treatment fruits in the two harvests evaluated. The parceled application of AVG (62.5 mg L^{-1} + 62.5 mg L^{-1}) did not differ from the control for the total phenolic compound content in the skin in Harvest 1 but reduced in Harvest 2. The reduction in total phenolic compound content in fruits treated with AVG was also observed by Ozturk et al. [33] in plums, Ozturk and Kucuker [34] and Yildiz et al. [35] in sweet cherries, and Ozturk et al. [7] in 'Braeburn' apples. According to Ozturk et al. [33], the decrease in the content of bioactive compounds by AVG may be due to inhibition of the synthesis of ethylene, because the stimulus to the production of ethylene has a consequence on the activation of the metabolism of phenylpropanoids in vegetables, due to the increase in the activity of the enzyme phenylalanine ammonia-lyase (PAL), which regulates the synthesis of phenolic compounds [36]. Khan et al. [37] indicate that the decrease in total phenolic compound content in fruits treated with AVG is related to the inhibition of the normal production of free radicals during the respiratory process and, consequently, the non-activation of secondary metabolism.

In Harvest 1, fruits of the plants that received the pre-harvest application of AVG in a single dose presented a lower total antioxidant activity content in the skin (ABTS and DPPH methods) than the control, while the application of parceled AVG did not differ from the control (Figure 2). In Harvest 2, any form of application of AVG provided a reduction in total antioxidant activity by the DPPH method, while total antioxidant activity by the ABTS method's reduction concerned the control only with the pre-harvest application of AVG in a single dose. Ozturk et al. [7] and Ceymann et al. [15] also observed a reduction in total antioxidant activity on 'Braeburn' apple skin that received a single dose application of AVG (100; 300; 500 mg L^{-1}), sprayed four weeks before the estimated harvest date. Lower total antioxidant activity in apples that received a pre-harvest application of AVG in a single dose reflects the lower total phenolic compound content, considering the positive and highly significant correlation between the total phenolic compound and total antioxidant activity (Table 1).

In Harvest 1, there was a reduction in anthocyanin content in fruits that received the pre-harvest application of AVG in a single dose, while apples that received the pre-harvest application of AVG in parcel showed no change in anthocyanin content compared to the control treatment fruits (Figure 2). In Harvest 2, any form of application of AVG showed a reduction in anthocyanin content compared to the fruits of the control treatment; however, the pre-harvest application of AVG in a single dose showed an even greater reduction when compared to the fruits that received a parceled application of AVG. The reduction in anthocyanin content in apples that received an application of AVG [single dose (100; 300; 500 mg L^{-1}), sprayed four weeks before the estimated harvest date] was also reported by Ozturk et al. [7] in 'Braeburn' apples. According to Awad et al. [38] and Li et al. [39], ethylene triggers the expression of genes from the biosynthesis of anthocyanin, because this phytohormone leads to the activation of secondary metabolism as a result of the increased activity of PAL, while AVG inhibits or delays its expression due to a reduction in the rate of ethylene production and, consequently, a lower concentration of anthocyanin in the fruit. Additionally, the use of anti-hail nets, by virtue of their shading effect, reduces the incident sunlight and can negatively impact the accumulation of anthocyanin on the epidermis (responsible for the red skin color) [20,21,40], presenting a negative synergistic effect with AVG.

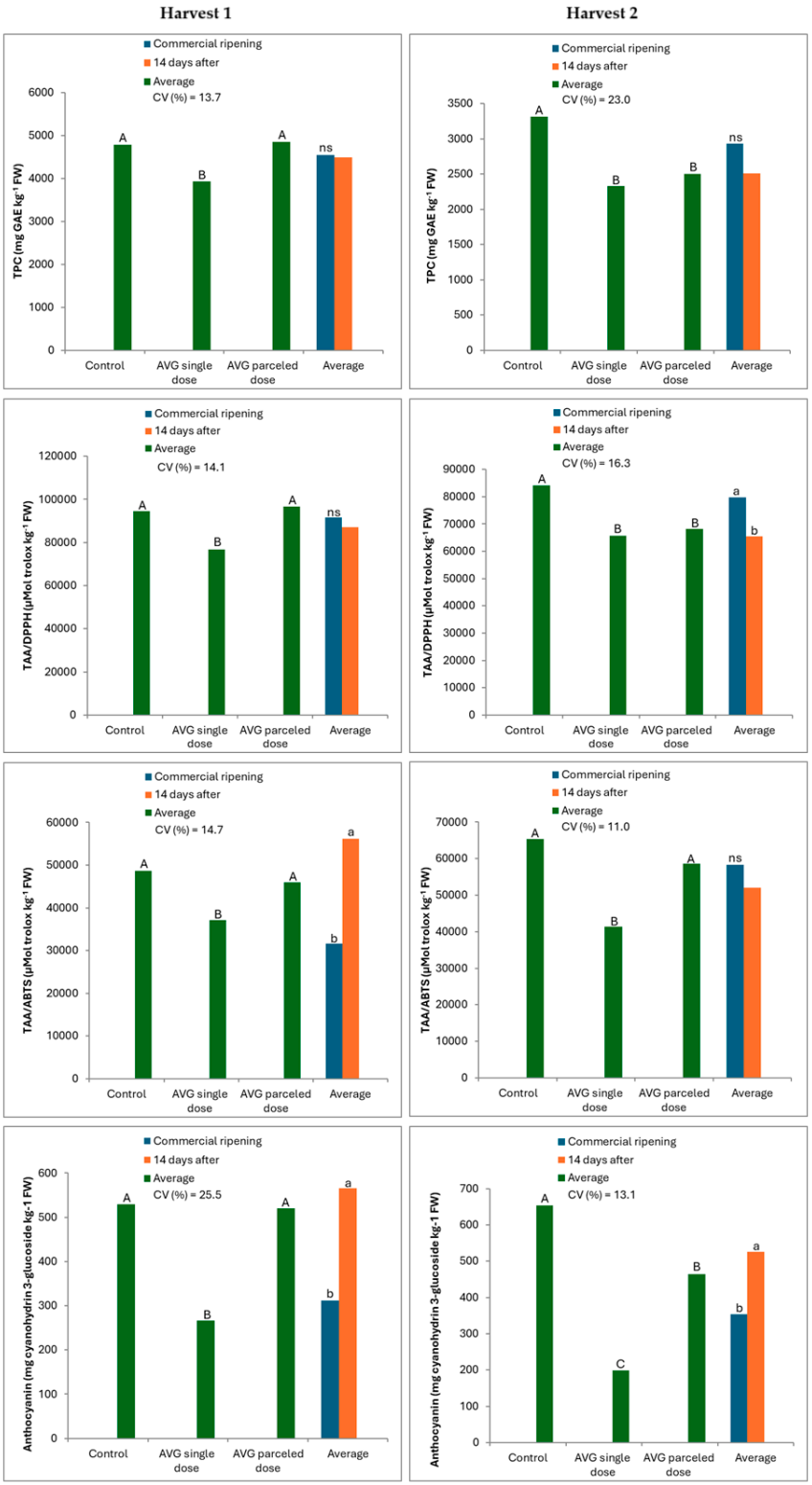

Figure 2. Total phenolic compound contents (TPC; mg GAE kg^{-1} FW), total antioxidant activity (TAA; DPPH and ABTS methods; and µMol trolox kg^{-1} FW), and anthocyanin content (mg cyanohydrin 3-glucoside kg^{-1} FW) in 'Baigent' apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey's test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

The application of AVG in a single dose reduced the concentration of chlorogenic acid and epicatechin in the skin, while the parceled application of AVG did not influence its concentration compared to the fruits of the control treatment (Figure 3). The lower content of chlorogenic acid and epicatechin in apples treated with AVG in a single dose may be related to the inhibition of ethylene synthesis, while the lower activation of the phenylpropanoid metabolism in the fruit may be because of the reduction in activity of the PAL enzyme, which regulates the synthesis of all phenolic compounds [33]. Thus, AVG can affect individual classes of phenolic compounds, reducing the concentrations of polyphenols in apples treated with AVG. On the other hand, any form of pre-harvest application of AVG has increased the content of floridizine and catechin in the skin, compared to the fruits of the control treatment. The quercetin content in the skin increased with the pre-harvest application of AVG in a single dose compared to the control treatment. In contrast, the parceled application of AVG did not differ among the other treatments. Kucuker et al. [41], when evaluating the whole fruit, also observed a lower chlorogenic acid content in plums that received a pre-harvest application of AVG. Ozturk et al. [33] observed a reduction in the content of chlorogenic acid and quercetin in plums, evaluated throughout the fruit treated with the highest dose of AVG (200 mg L^{-1}) but not with the lowest dose (100 mg L^{-1}). Therefore, the effect of AVG can be dose-dependent. In 'Huangguan' pears, AVG application decreased the malondialdehyde (MDA) content and polyphenol oxidase (PPO) activity, delayed the peak of chlorogenic acid (CGA) content in the core tissue, and significantly inhibited the expression of genes such as ACC synthase, ACC oxidase, ethylene receptors, ethylene response factor, phenylalanine ammonia lyase, cinnamate 4-hydroxylase, 4-hydroxycinnamoyl-CoA ligase, hydroxycinnamoyl-CoA shikimate hydroxycinnamoyl transferase, and polyphenol oxidase, as well as phospholipase D and lipoxygenase. [42]. In 'Cripps Pink' apples skin tissues, different from the present work, the application of AVG did not influence the content of chlorogenic acid, epicatechin, floridizine, catechin, and quercetin [43]. The differences observed between the different works and the present study may be related to the species and cultivars used in the study. The chemical composition of fruits, including phenolic compounds, varies according to the cultivar, environmental conditions, cultural practices, nutrient contents, and fruit ripening stage [17,19,44]. In addition, the methods of analysis used and the type of tissue sampled (skin, flesh, or whole fruit) can also produce, among the various research works, different results for phenolic compounds [17,45].

Individual phenolic compounds have a premium contribution in the antioxidant activity [12,46], the phenolic acids being the main acids responsible for the antioxidant activity in apples, with an emphasis on chlorogenic acid [47]. However, other studies consider glycosylated quercetin as one of the phenolic compounds, which contribute most to the antioxidant activity in apples, because it has structural advantages over other molecules [48,49], being one of the most effective compounds in all antioxidative parameters [13]. In the present study, the total antioxidant activity was superior in fruits in the control treatment (Figure 2), which had the lowest quercetin content and the highest chlorogenic acid content (Figure 3).

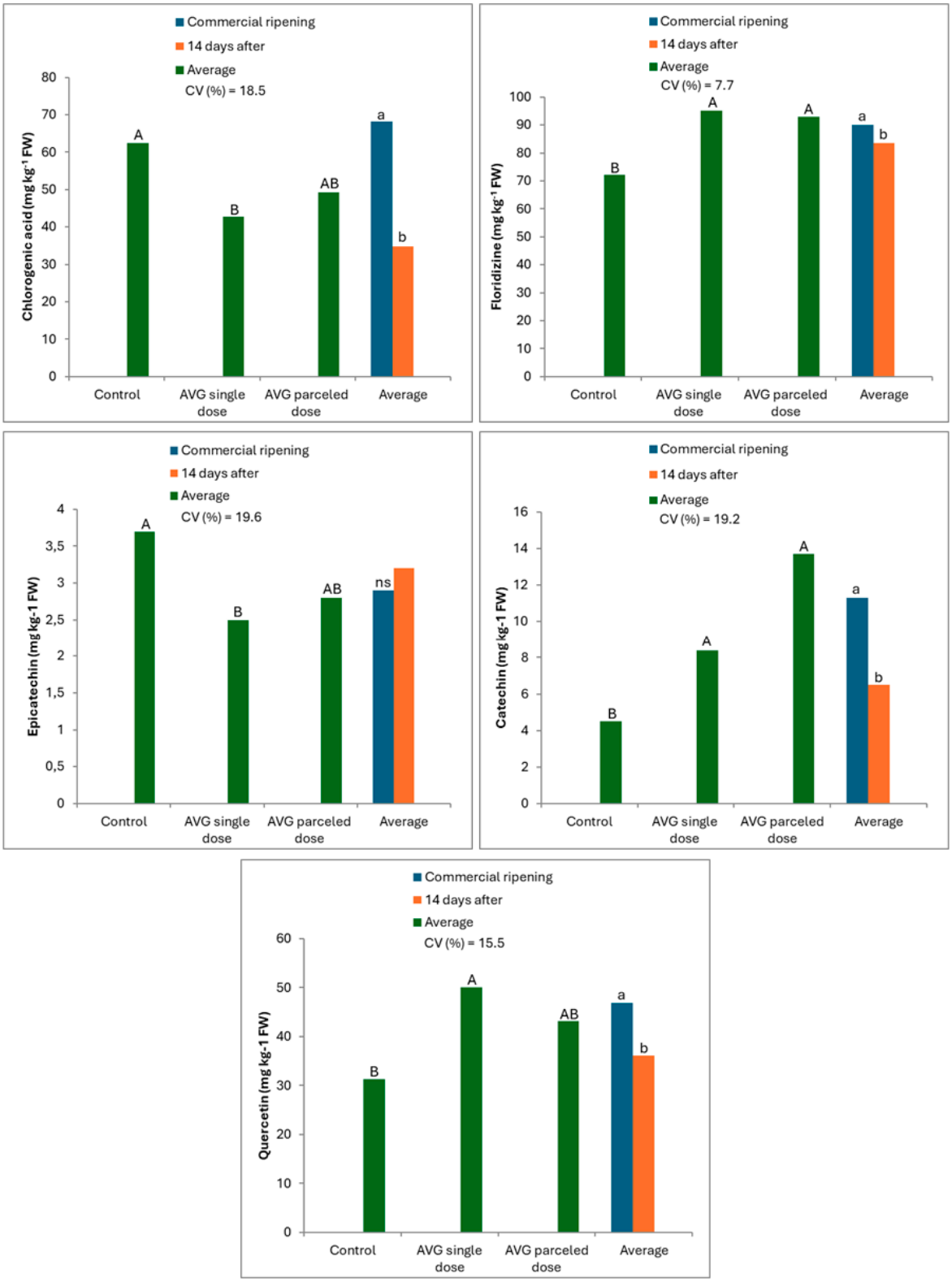

Figure 3. Chlorogenic acid, floridizine, epicatechin, catechin, and quercetin (mg kg^{-1} FW) levels in 'Baigent' apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates in Harvest 2. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey's test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

With the harvest delay, there was an increase in the total phenolic compound and total antioxidant activity values (DPPH and ABTS methods) in the flesh for Harvest 1. In Harvest 2, there was a reduction in the total phenolic compound and total antioxidant activity content by the ABTS method in the flesh but not in the total antioxidant activity by the DPPH method with a delay in harvesting (Figure 1). In the skin, no change in the total phenolic compound content was observed with the harvest delay in the two harvests evaluated. In Harvest 1, by the DPPH method, no difference was observed in the total antioxidant activity in the skin between the two harvests, while by the ABTS method, there was an increase in the total antioxidant activity in the shell with the delay in harvesting. In Harvest 2, there was a reduction in total antioxidant activity by the DPPH method in the skin with the harvest delay but not the total antioxidant activity by the ABTS method (Figure 2). However, the occurrence and content of phenolic compounds and antioxidant capacity vary between cultivars [17], orchard management [18], sun exposure, and stage of ripening [50].

The anthocyanin content increased with the delay of harvest in the two harvests evaluated (Figure 2), indicating an increase in the red skin color of the fruits, as also observed by Whale et al. [43] and Li et al. [51]. However, with the harvest delay, there was a reduction in the content of chlorogenic acid, floridizine, catechin, and quercetin but not of epicatechin (Figure 3). These different phenolic compounds are intermediate in the biosynthesis route of anthocyanin, and their reduction may be related to the increase in anthocyanin content.

3.2. After Cold Storage (CS)

In the flesh, after four months of cold storage, no difference was observed between pre-harvest treatments for the total phenolic compound and total antioxidant activity values (DPPH and ABTS methods) in the two harvests evaluated (Figure 4). Kucuker et al. [45] also did not observe the effect of AVG on these variables in the plum flesh after cold storage. These results agree with the findings of Aglar et al. [52], who reported that the application of AVG had no significant effect on the total antioxidant activity or the total phenolic compound content in jujube fruits after cold storage. According to Rotili et al. [53], total antioxidant activity in fruits results from the action of a variety of compounds that are degraded or synthesized during storage in response to biotic and abiotic stresses.

In the skin, during Harvest 1, treatment with AVG, regardless of the form of application, caused a reduction in the total phenolic compound content compared to the fruits of the control treatment (Figure 5). In Harvest 2, only the pre-harvest application of AVG in a single dose provided a lower total phenolic compound content compared to the control. The pre-harvest parceled application of AVG presented intermediate values not differing from the other treatments. Therefore, the pre-harvest application of AVG in a split dose showed a less-pronounced negative effect than the application of AVG in a single dose on the total phenolic compound content in the skin of fruits kept in cold storage. Karaman et al. [54] and Ozturk et al. [34] also observed a lower total phenolic compound content in plums that received a pre-harvest application of AVG after the cold storage period. The lower total phenolic compound content in ap-ples treated with AVG may be related to a reduced ethylene production rate in the fruit. According to Picoli et al. [55], the increase in the rate of ethylene production in-duces the activity of the enzyme PAL, which regulates the synthesis of phenolic com-pounds, thus AVG can reduce the content of the total phenolic compound by reducing the synthesis of ethylene.

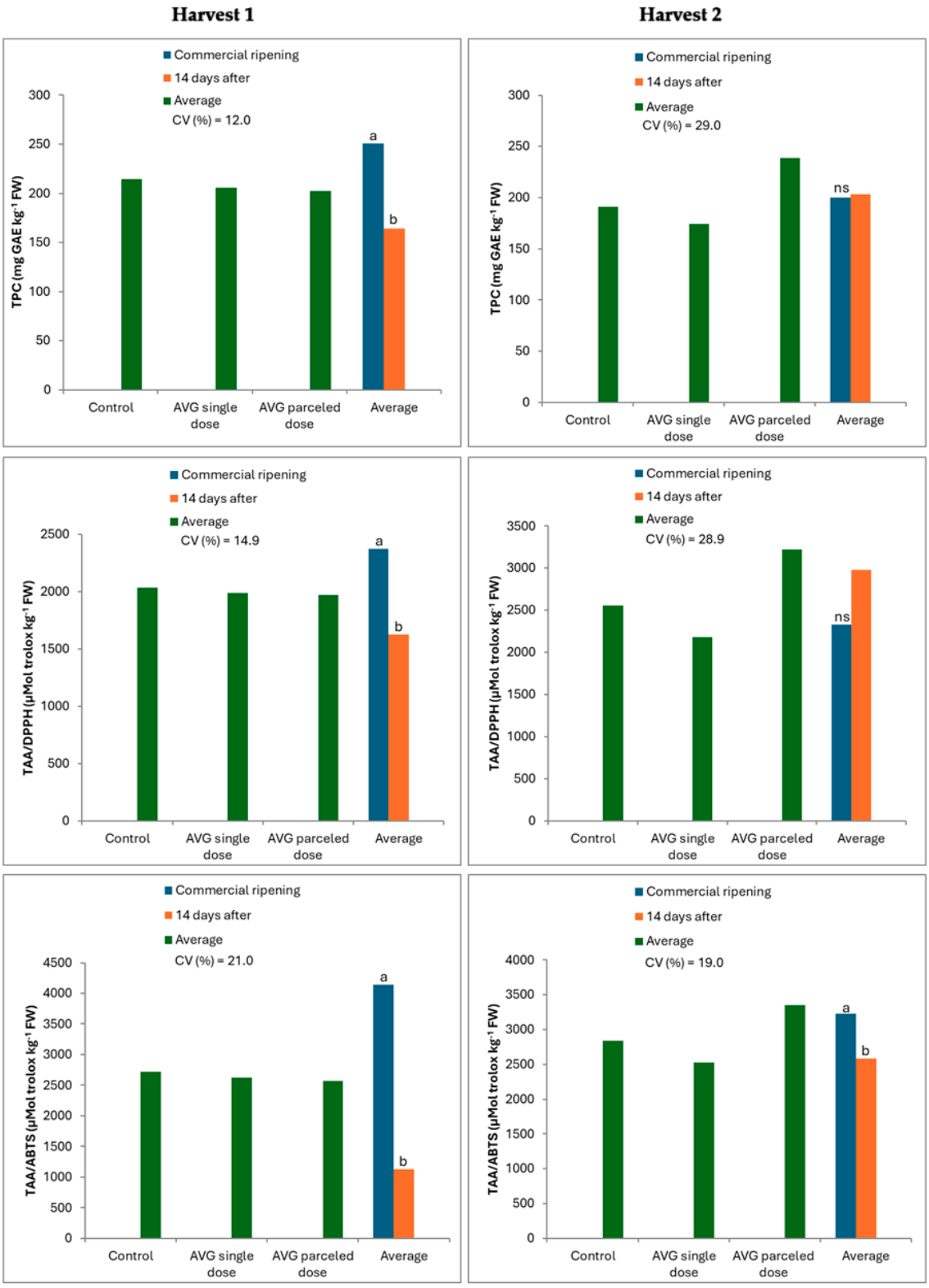

Figure 4. Total phenolic compound contents (TPC; mg GAE kg^{-1} FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and µMol trolox kg^{-1} FW) in 'Baigent' apple flesh, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH). Treatment averages comparing harvest dates, not followed by the same lower-case letter, differ by Tukey test ($p < 0.05$). ns: non-significant difference between treatments for average data of the two harvest dates.

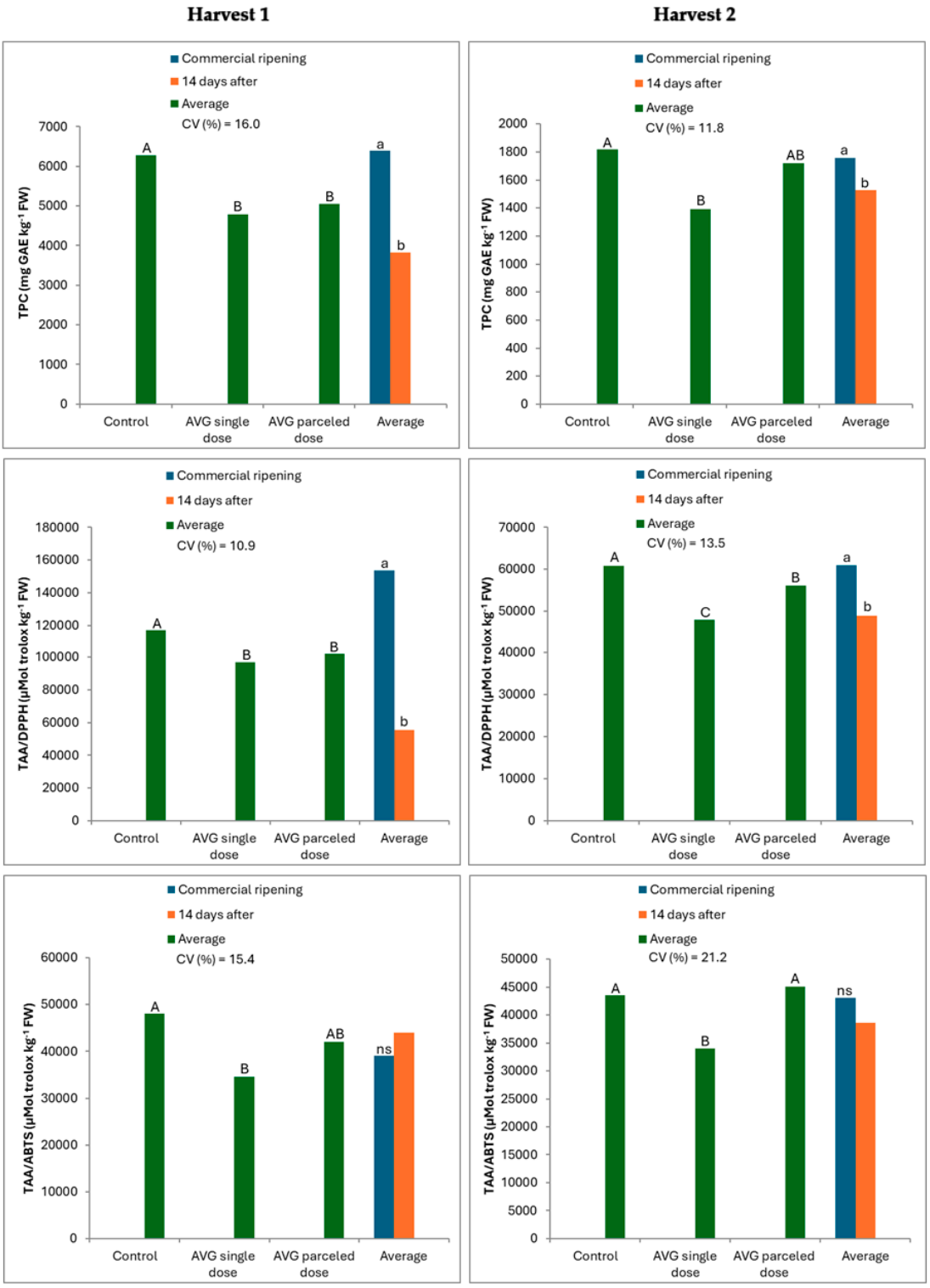

Figure 5. Total phenolic compound contents (TPC; mg GAE kg^{-1} FW) and total antioxidant activity (TAA; DPPH and ABTS methods; and µMol trolox kg^{-1} FW) in 'Baigent' apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH). Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey's test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

In the skin, for both seasons, any form of pre-harvest application of AVG reduced the total antioxidant activity quantified through the DPPH method, compared to the fruits of the control treatment (Figure 5). However, in Harvest 2, the reduction in total antioxidant activity quantified by the DPPH method in single-dose AVG-treated apples was greater than in apples that received a parceled application of AVG. For the total antioxidant activity quantified by the ABTS method, only the pre-harvest application of single-dose AVG reduced the total antioxidant activity relative to the control in both seasons. These results indicate that the parceled application of AVG has a less-intense negative impact than single-dose AVG after cold storage, presenting similar or closer values to the fruits of the control treatment. Karaman et al. [54] also observed lower total antioxidant activity in plums that received the pre-harvest application of AVG after the cold storage period than those of the control treatment.

There was interaction between the pre-harvest treatment factors and harvest dates for the chlorogenic acid content (Table 2). In the commercial ripening, the pre-harvest application of AVG in a single dose and parcel showed lower chlorogenic acid content compared to the fruits of the control treatment. In the 14 days after harvest, only the application of AVG in a parceled dose showed a lower chlorogenic acid content com-pared to the fruits of the control treatment. Ozturk et al. [34] also observed a lower chlorogenic acid content in plums treated with AVG after the cold storage period.

Table 2. Chlorogenic acid (mg kg^{-1} FW) contents of 'Baigent' apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH) for Harvest 2.

Treatments	Commercial Ripening	14 Days After	Average
	Chlorogenic acid		
Control	128.6 Aa	59.2 Ab	-
AVG single dose (125 mg L^{-1}/30 DBEHD *)	67.3 Ba	59.4 Aa	-
AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)	35.4 Ca	34.6 Ba	-
Average	-	-	
CV (%)	17.5		

* DBEHD: Before the estimated harvest date. Averages not followed by the same letter, upper case in the columns and lower case in the rows, differ by Tukey test ($p < 0.05$).

There was no effect of the AVG pre-harvest application on the floridizine content (Figure 6). For catechin, only the split application of AVG reduced its content compared to the fruits of the control treatment. Lower quercetin content was observed in the fruits that received an application of AVG, independent of the form. The results of this work are in accordance with those obtained in plums, where there was a reduction in the concentration of catechin and quercetin with the application of AVG after cold storage [34].

In Harvest 1, there was a reduction in the total phenolic compound and total antioxidant activity content in the flesh with the harvest delay. In Harvest 2, there was no change in the total phenolic compound content and in the total antioxidant activity by the DPPH method, while in the total antioxidant activity by the ABTS method, there was a reduction with the harvest delay (Figure 4). In the skin, a reduction in the total phenolic compound content and in the total antioxidant activity by the DPPH method was observed with the harvest delay, while no change in the total antioxidant activity by the ABTS method was observed in the two seasons evaluated (Figure 5). There was a reduction in floridizine and chlorogenic acid concentrations in the fruits of the control treatment with a harvest delay,

while in fruits with AVG applications, there was no effect of the harvest date on chlorogenic acid concentration. On the other hand, an increase in the quercetin concentration was observed, and no difference was observed for the catechin concentration with a harvest delay (Figure 6).

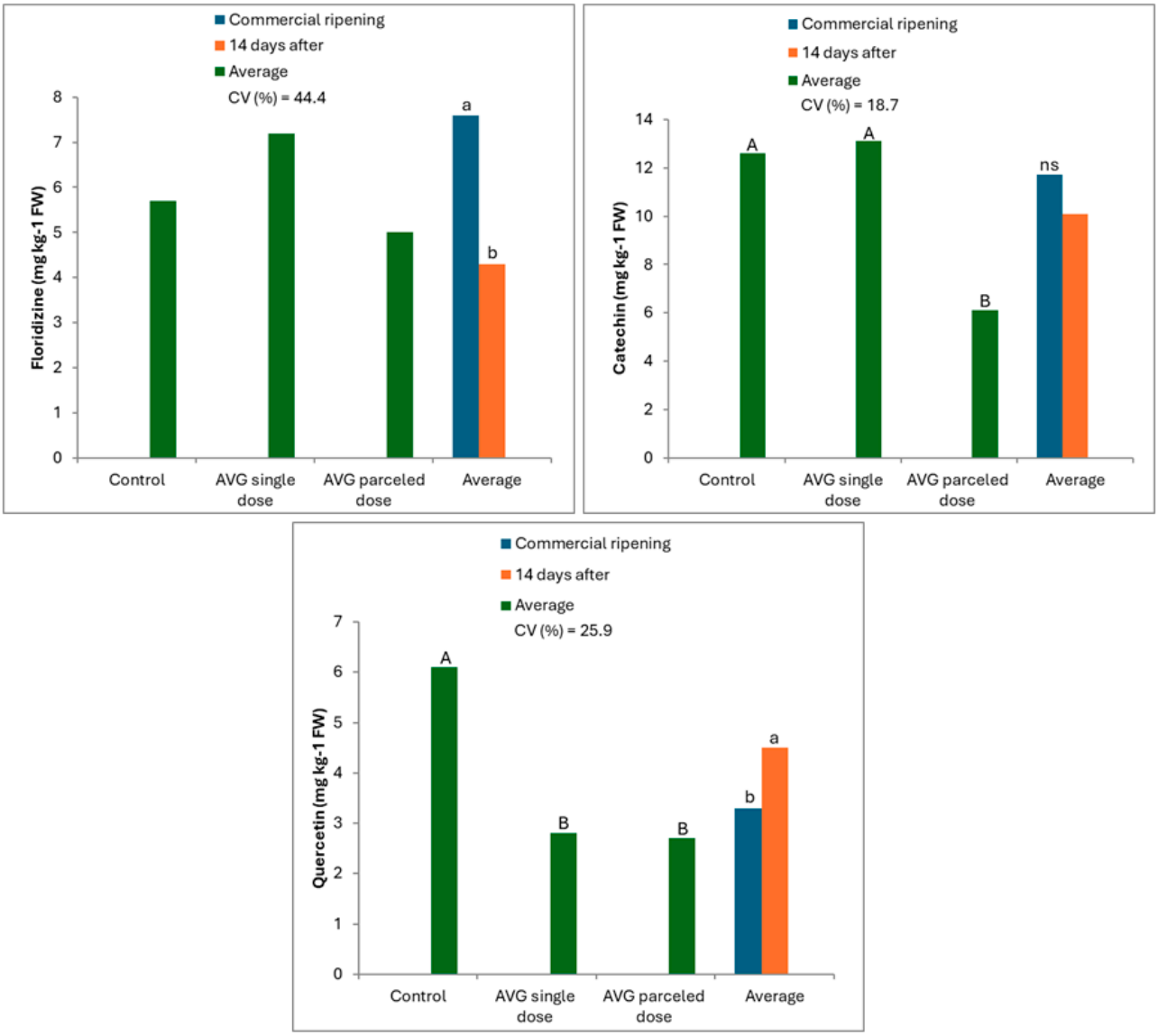

Figure 6. Floridizine, catechin, and quercetin (mg kg^{-1} FW) contents of 'Baigent' apple skin, in response to pre-harvest application of aminoethoxyvinylglycine (AVG) (AVG single dose (125 mg L^{-1}/30 DBEHD—before the estimated harvest date) and AVG parceled dose (62.5 mg L^{-1} + 62.5 mg L^{-1}/30 and 20 DBEHD)) and two harvest dates, evaluated after four months of cold storage, followed by seven more days in ambient conditions (20 ± 1 °C and 65 ± 5% RH) for Harvest 2. Treatment means that, when comparing harvest dates, are not followed by the same lowercase letter, and treatment means with or without AVG application that are not followed by the same uppercase letter, differ according to Tukey's test ($p < 0.05$). ns: non-significant difference between treatments, based on the average of the two harvest dates.

4. Conclusions

This study demonstrated that the pre-harvest application of AVG in a single dose reduces total phenolics, anthocyanins, and antioxidant activity in the skin of 'Baigent' apples grown under anti-hail nets, both at harvest and after cold storage. In contrast, the split AVG application resulted in less pronounced reductions and values closer to the

control, particularly for phenolics and anthocyanins. These findings suggest that split AVG applications may be a preferable strategy for preserving the nutritional and functional quality of apples destined for storage. Future studies should investigate the economic feasibility of multiple applications and assess whether these results are consistent under open-field conditions without anti-hail nets.

Author Contributions: Conceptualization, C.S., C.V.T.d.A. and C.A.S.; methodology, C.S., I.d.O.C., F.J.H., M.A.M. and M.C.S.; formal analysis, C.S., I.d.O.C., F.J.H., M.A.M. and M.C.S.; investigation, C.S., I.d.O.C., C.A., N.M.d.S., F.J.H., M.A.M., M.C.S., C.V.T.d.A. and C.A.S.; resources, C.S., N.M.d.S. and C.A.; data curation, C.S., C.A.S. and C.V.T.d.A.; writing—original draft preparation, C.S.; writing—review and editing, N.M.d.S., and C.A.; supervision, C.A.S. and C.V.T.d.A.; project administration, C.A.S. and C.V.T.d.A.; funding acquisition, C.A.S. and C.V.T.d.A. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Nitric Oxide in Controlled Atmosphere Storage of 'Fuji Mishima' Apples

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Abstract

'Fuji' apples require long-term storage to ensure year-round supply, and controlled-atmosphere (CA) technology is widely used to preserve their quality and reduce postharvest losses. Nitric oxide (NO), a natural signaling molecule in plants, has shown potential to delay ripening and reduce physiological and pathological disorders during fruit storage. This study evaluated the effect of nitric oxide (NO) treatment during controlled-atmosphere (CA) storage on the postharvest quality of 'Fuji Mishima' apples. Apples were stored for 8 months at 1 kPa O_2 +< 0.5 kPa CO_2, 1.0 ± 0.2 °C, and 94 ± 2% RH. The treatments consisted of a control (without NO) and five NO application regimes: 5 μL L^{-1} applied at the beginning of storage; 5 μL L^{-1} applied both at the beginning and end; 5 μL L^{-1} applied every 30 days; 10 μL L^{-1} applied at the beginning; and 10 μL L^{-1} applied both at the beginning and end of storage. All NO treatments delayed ethylene production and reduced its levels after 4 days under ambient conditions compared to the control. However, NO had no effect on flesh firmness, soluble solids content, titratable acidity, peel color, or flesh browning. Repeated NO applications (5 or 10 μL L^{-1}) increased peel yellowing. Treatment with 5 μL L^{-1} applied every 30 days increased decay incidence. Phenolic compounds in the flesh were unaffected, while in the peel, they decreased with 10 μL L^{-1}. Overall, NO application in CA storage of 'Fuji Mishima' apples did not maintain fruit quality and, in some cases, increased peel yellowing and decay.

Keywords: *Malus domestica* Borkh.; ethylene; decay; flesh browning; postharvest; ripening; oxidative stress

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1. Introduction

The Brazilian apple harvest occurs over a short period, mainly from February to April, resulting in a large portion of the yield needing to be stored in order to regulate the supply of the fruit throughout the year. Controlled-atmosphere (CA) storage is the leading technology for preserving apple during long-term storage [1].

Despite the advantages of CA, apples from the 'Fuji' group are susceptible to damage caused by high CO_2 partial pressure (>0.5 kPa) during CA storage. Therefore, it is recommended to adopt <0.5 kPa CO_2 during CA storage to avoid browning damage [2]. Additionally, the incidence of decay, especially in prolonged storage, can account for up to 80% of postharvest losses of 'Fuji' apples [3]. Therefore, additional technologies are required to complement CA storage and maintain fruit quality.

Nitric oxide (NO) is a gaseous molecule naturally produced by plants which participates in various physiological processes, including fruit ripening, with positive effects on postharvest quality regarding flavor preservation, reduction of disease development, and physiological disorders [4]. In the postharvest period, NO has an antagonistic effect on ethylene production by interfering with the production of its precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), which results in lower activity of the enzymes ACC synthase and ACC oxidase, and consequently inhibits the activity of enzymes responsible for fruit softening [5]. NO also increases the ability to metabolize reactive oxygen species (ROS) through the induction of antioxidant metabolism enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO), resulting in the maintenance of overall antioxidant capacity [4].

The postharvest application of NO, associated with CA storage, has already shown positive results in various fruits, including 'Baigent' [6] and 'Cripps Pink' apples [7,8]. NO application has also proven effective in reducing surface browning of minimally processed 'Granny Smith' apples [9], 'Royal Gala', 'Golden Delicious', 'Sundowner', 'Fuji', and 'Red Delicious' apples [10].

The postharvest metabolic pathways influenced by NO remain only partially understood. It is known that the effectiveness of using NO may change depending on factors such as cultivar, epigenetic modifications, storage conditions, and others, such as concentration, time, and moments of application, influencing its action [11–13]. Additionally, NO also has the ability to interact with other molecules, resulting in the alteration of fruit responses [11]. During postharvest storage, nitric oxide may interact with a variety of molecules, including ethylene, reactive oxygen species (ROS), hydrogen sulfide (H_2S), melatonin, and antioxidant-related enzymes. Interaction with ethylene leads to inhibition of its biosynthesis, thereby delaying ripening and mitigating physiological disorders. NO modulates the antioxidant system by inducing enzymes such as catalase, peroxidase and superoxide dismutase, thus enhancing the fruit's tolerance to oxidative stress. Additionally, NO's interplay with signaling molecules like H_2S and melatonin further influences stress response and maintenance of fruit quality during storage [14,15]. Currently, there is no information of NO application in 'Fuji' apples group during CA storage. In this context, the objective of this study was to evaluate the effect of two doses of NO, applied at different moments during CA storage, on 'Fuji Mishima' apples quality maintenance.

2. Materials and Methods

2.1. Plant Material and Experimental Details

The experiment was conducted with 'Fuji Mishima' apples harvested during the 2022/2023 season from a commercial orchard located in the municipality of São Joaquim, Santa Catarina, Brazil (28°16′01.7″ S; 49°54′17″ W; 1400 m altitude, Cfb climate according to the Köppen classification). The 'Fuji Mishima' apple trees used in this study were 12 years old and grafted on the vigorous rootstock 'Marubakaido' with an M.9 interstem (Marubakaido/M.9 combination). This rootstock combination is commonly used in commercial orchards in southern Brazil, particularly in São Joaquim (SC). The orchard was established with a planting spacing of 4.0 m between rows and 1.2 m between trees within the row. The canopy training system used was the central leader, which is commonly

adopted in commercial apple orchards in southern Brazil. After harvest, apples were transported to the laboratory, sorted for uniform size and maturity, and fruit showing any mechanical or pathogen-related damage were discarded.

The fruit initially presented the following ripening attributes: background peel color with lightness (L*), chromaticity (C*), and hue angle of 70.5, 42.1, and 100.8°, respectively; flesh firmness of 74.2 N, soluble solids content of 15.8 °Brix, and titratable acidity of 0.38% malic acid, with an average iodine-starch index of 4.5 (scale 1–5).

For NO treatment, the fruit were placed in experimental micro-chambers (80 L) under CA conditions of 1 kPa O_2 + < 0.5 kPa CO_2, at a temperature of 1.0 ± 0.2 °C and relative humidity of 94 ± 2%, for 8 months. The evaluated treatments were as follows: control (without NO); 5 μL L^{-1} NO applied at the beginning of storage; 5 μL L^{-1} NO applied at the beginning and end of storage; 5 μL L^{-1} NO applied at the beginning and every 30 days during storage; 10 μL L^{-1} NO applied at the beginning of storage; and 10 μL L^{-1} NO applied at the beginning and end of storage. NO concentrations (5 and 10 μL L^{-1}) were selected according to previous studies conducted with other apple cultivars such as Baigent and Cripps Pink [6,7].

The application of NO in the micro-chambers was performed using a mixture of NO + N_2 gas (standard mixture containing 1000 μL L^{-1} of NO + N_2 in balance, White Martins®, Brazil) from high-pressure cylinders. The initial application was carried out when microchambers O_2 partial pressure reached 1 kPa (4 days after storage), every 30 days from the first application, or at the end of storage, according to the treatment. The last NO application, for treatments with reapplication every 30 days and at the end of storage, occurred 5 days before the finished of the storage period.

The O_2 and CO_2 partial pressure were monitored daily using an automated system for monitoring and controlling CA (Multiplex Isosoft, Isolcell, Laives, Italy). The oxygen consumption due to fruit respiration were corrected by injecting O_2 from high-pressure cylinders. For CO_2 absorption, hydrated lime was placed inside the micro-chamber in the proportion of 1 kg of hydrated lime per 20 kg of fruit. The storage temperature was monitored daily using mercury thermometers (5100, Incoterm®, Porto Alegre, Brazil).

2.2. Evaluation of Fruit Quality Attributes

The fruits were evaluated upon opening the chambers for ethylene production rate, background peel color, and incidence of decay. After 7 days of shelf life (20 ± 1 °C; RH of 65 ± 5%) flesh firmness, soluble solids content (SSC), titratable acidity (TA), incidence of flesh browning, and decay were also performed. The ethylene production rate was evaluated every 2 days during shelf life, from the exit of storage (D0), until 6 days of shelf life (D2, D4, and D6, respectively).

After 7 days of shelf life, samples of flesh and peel were separated to determine phenolic compounds and antioxidant activity using the ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging methods. The samples were frozen in liquid nitrogen immediately after fruit cutting. The skin samples were processed and kept fresh in an ultrafreezer (Thermo Fischer Scientific, 910, Waltham, MA, USA) at −50 °C until analysis, while the flesh samples were freeze-dried in a freeze dryer (LH, Terroni®, São Carlos, Brazil). The freeze-dried samples were processed in a vibratory ball mill (BM 500, Anton Paar®, Graz, Austria) and stored in a desiccator until analysis. The analyses were carried out within 30 days after freeze-drying (lyophilization) of the samples.

To determine ethylene production (ηmol C_2H_4 kg^{-1} s^{-1}), approximately 1 kg of fruit per replicate were placed in hermetically sealed glass jars (6.1 L), for 120 min. Then, air samples were withdrawn from the headspace of the jars, using plastic syringes of 1 mL,

and injected into a gas chromatograph with flame ionization detection (GC-FID) (Clarus 580, PerkinElmer®, Shelton, CT, USA), equipped with a 3 m Porapak N column (mesh 80–100). The temperatures of the column, detector, and injector were 70, 250, and 130 °C, respectively. The gas flow rates were 70, 30, and 300 mL min^{-1} for nitrogen, hydrogen, and synthetic air, respectively. Nitrogen gas was used as the mobile phase.

Skin background color was measured on the greenest side of the fruit peel using a CR-400 colorimeter (Konica, Minolta®, Tokyo, Japan), on the L* (lightness), C* (chromaticity), and h° (hue) scale.

Flesh firmness was determined on the equatorial region of each fruit after removing a small portion of the epidermis, at two opposite points on each fruit, using an electronic penetrometer (Güss Manufacturing Ltd., Cape Town, South Africa), equipped with an 11 mm diameter probe, and the results were expressed in Newtons (N).

For the SSC and TA, samples of flesh and skin were taken from transverse slices cut from the equatorial region of the fruits, which were then crushed in an electric centrifuge (PG 710/G5, Champion Juicer®, Lodi, CA, USA). The SSC was evaluated using a digital precision refractometer with automatic temperature measurement and compensation to 20 °C (PR201α, Atago®, Tokyo, Japan) using 0.5 mL of juice, with the results expressed in °Brix. The TA was determined using an automatic titrator (TitroLine Easy, Schott Instruments®, Weilheim, Germany) using a 5 mL juice sample diluted in 45 mL of distilled water, which was titrated with a 0.1 N NaOH solution until pH 8.1, with the results expressed as % malic acid.

To evaluate flesh browning (results expressed as %), the fruits were cut in a transverse section at the equatorial region and observed for the presence of browning (i.e., visually presented any type of browning). The incidence of decay was evaluated by counting the number of apples exhibiting fungal infection symptoms with lesions larger than 5 mm.

To obtain the extracts for the analysis of total phenolic compounds and antioxidant activity, 0.5 g of freeze-dried flesh and 1 g of fresh skin were added to 10 mL of 80% methanol, followed by homogenization in an Ultra-Turrax (SilentCruscher M, Heidolph, Schwabach, Germany), and left to rest protected from light for 60 min at room temperature. The extracts were then centrifuged at 15,000 rpm for 15 min at 4 °C in a centrifuge (CR22N, Hitachi, Tokyo, Japan), subsequently filtered, and the supernatant collected.

The determination of phenolic compound content was performed using the Folin-Ciocalteau reagent (Sigma Aldrich, Darmstadt, Germany) adapted from Roesler et al. [16]. The standard curve was obtained with gallic acid. For analysis, 0.25 µL of extract, 200 µL of distilled water, and 25 µL of 2 N Folin-Ciocalteau reagent were added to microplates. The mixture was allowed to stand for 5 min, and then 25 µL of 10% sodium carbonate was added. Readings were taken after 60 min using a microplate reader (EnSpire, PerkinElmer®, Shelton, CT, USA) at 725 nm. The results were expressed in milligrams of gallic acid equivalent per gram of fresh (FM) or dry mass (DM) (mg GAE g^{-1} FM for peel or mg GAE g^{-1} DM for flesh).

The determination of antioxidant capacity by the ABTS free radical scavenging method was performed according to Rufino et al. [17], with modifications. In microplates, 10 µL of extract and 290 µL of ABTS radical were added and kept in the dark for 6 min, and then the absorbance was read at 734 nm using a microplate reader (EnSpire, PerkinElmer®, Shelton, CT, USA). The calibration curve was constructed with standard Trolox solutions, and the results were expressed in mg TEAC g^{-1} FM for skin or mg TEAC g^{-1} DM for flesh.

The determination of antioxidant capacity by the DPPH free radical scavenging method was conducted according to the method described by Rufino et al. [18], with modifications. In microplates, 40 µL of extract from the samples were added, followed by 260 µL of DPPH solution, and allowed to react for 30 min in the dark. Reading were

then taken at a wavelength of 525 nm using a microplate reader (EnSpire, PerkinElmer®, Shelton, CT, USA). The results were expressed as a percentage of DPPH radical scavenging (% DPPH inhibition).

2.3. Experimental Design and Statistical Analysis

The experimental design was completely randomized, with four replicates, each consisting of 30 fruits. The data were analyzed using RStudio 3.5.0 software (Posit, Boston, MA, USA). Shapiro–Wilk normality tests of residuals ($p < 0.05$) and Bartlett's homogeneity of variance tests ($p < 0.05$) were first employed. Normal data were then subjected to analysis of variance (ANOVA), and the means were compared using the Scott–Knott test ($p < 0.05$). Data expressed as percentages were previously transformed into $\arcsin\sqrt{(x + 0.1)/100}$.

3. Results and Discussion

After 8 months of storage, the ethylene production rate of 'Fuji Mishima' apples showed no differences between treatments upon chamber opening, as well as at 2 and 6 days of shelf life (Figure 1). After 4 days of shelf life, control fruit reached the ethylene production peak, a rate about 1.5 times higher than those treated with different NO doses and frequencies. However, this effect of reduced ethylene production with NO application did not persist until 6 days under ambient conditions.

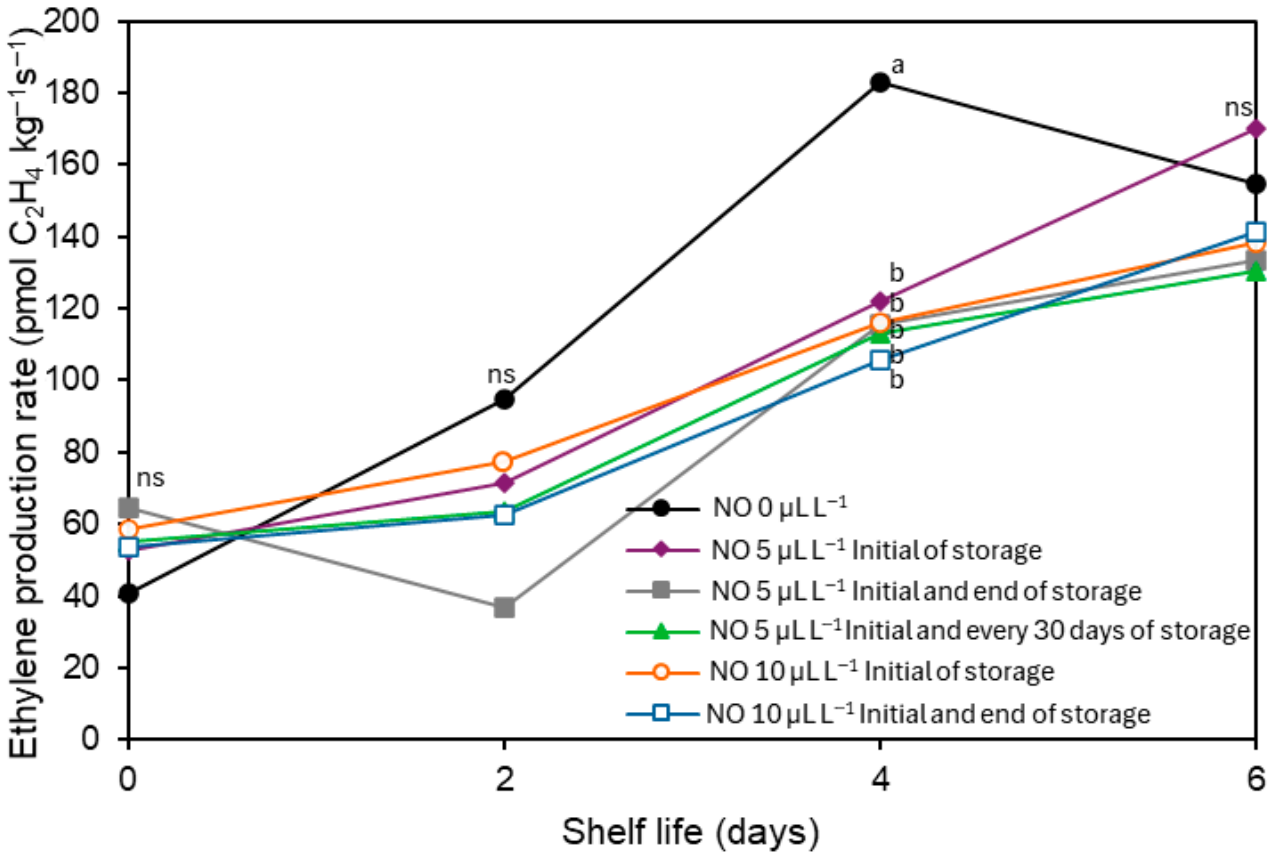

Figure 1. Ethylene production rate in 'Fuji Mishima' apples after 8 months of storage in a controlled atmosphere (1.0 kPa O_2 + < 0.5 kPa CO_2; 1.0 ± 0.2 °C; 94 ± 2% RH), upon chamber opening (Day 0), and after the subsequent 6 days of shelf life (20 ± 1 °C; 65 ± 5% RH), as a function of nitric oxide (NO) application in two doses (5 or 10 µL L^{-1}) and at different times during storage. Treatments followed by the same letter in the same evaluated day do not differ by the Scott–Knott test ($p < 0.05$). ns: not significant by ANOVA.

Similar results were observed for 'Cripps Pink' apples, where the application of NO at the beginning of CA storage was effective in reducing ethylene production at 4 days of shelf life [8]. However, at 6 and 7 days of shelf life, respectively, for 'Galaxy' and 'Cripps Pink', there was no effect from NO. In 'Galaxy' apples, the effect of NO on ethylene production was, at least in part, due to the lower activity of the ACC oxidase enzyme, a key enzyme in ethylene synthesis. NO acts by inhibiting ethylene synthesis through the formation of the ACC-oxidase-NO-ACC complex. Additionally, NO induces the reduction of ACC to 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC), decreases the activity of ACC synthase and ACC oxidase enzymes, and negatively regulates the expression of genes involved in the ethylene signaling pathway [11,19]. The results of this study demonstrate that NO application delayed the peak of ethylene production, corroborating the results obtained by Coser et al. [6] in 'Baigent' apples.

For the background peel color, differences between treatments were observed for the hue angle attribute upon exiting the chamber, and for L* after 7 days of shelf life (Table 1). Higher hue angle were observed in fruits not treated with NO, and in those treated with 5 and 10 μL L^{-1} NO at the beginning of storage, indicating a greener background peel color and, therefore, less mature fruits. However, this result did not persist after 7 days of shelf life, where the treatments did not differ from each other. After 7 days of shelf-life, the treatments with 5 and 10 μL L^{-1} NO at the beginning of storage and 10 μL L^{-1} NO applied at the beginning and end of storage, showed fruit with higher L* values than the other treatments. The NO application did not show benefits in maintaining the background color of 'Fuji Mishima' apples.

Table 1. Skin background color attributes of 'Fuji Mishima' apples after 8 months of storage in a controlled atmosphere (1.0 kPa O_2 + < 0.5 kPa CO_2; 1.0 ± 0.2 °C; RH of 94 ± 2%), upon chamber opening, and after 7 days of shelf life (20 ± 1 °C; RH of 65 ± 5%), as a function of nitric oxide (NO) application in two doses and at different times during storage. NO: nitric oxide.

Treatments		Skin Background Color		
NO Doses	**Period of Application**	**L***	**C***	**h°**
			Exit of the Chamber	
0 μL L^{-1}	No application	67.9 ^{ns}	37.0 ^{ns}	98.8 a
5 μL L^{-1}	Beginning	69.1	35.3	98.2 a
5 μL L^{-1}	Beginning and end	68.4	36.6	96.3 b
5 μL L^{-1}	Beginning + every 30 days	69.4	38.2	97.0 b
10 μL L^{-1}	Beginning	69.9	37.8	99.4 a
10 μL L^{-1}	Beginning and end	69.3	37.0	96.6 b
CV (%)		2.1	3.9	1.5
			7 days of shelf life	
0 μL L^{-1}	No application	69.0 b	42.1 ^{ns}	97.0 ^{ns}
5 μL L^{-1}	Beginning	70.8 a	39.8	96.0
5 μL L^{-1}	Beginning and end	67.7 b	40.2	95.0
5 μL L^{-1}	Beginning + every 30 days	69.7 b	41.9	96.5
10 μL L^{-1}	Beginning	71.7 a	39.8	98.4
10 μL L^{-1}	Beginning and end	71.9 a	40.8	95.3
CV (%)		2.2	3.2	2.4

Means followed by the same letter in the column do not differ by the Scott–Knott test ($p < 0.05$). ns: not significant by ANOVA.

Contrary to the present study, 'Baigent' apples, regardless of the dose and application time, after 7 days of shelf life, showed higher hue angle for fruit treated with NO compared

to the control, while no difference was observed in terms of L* [6]. A similar result was observed for 'Cripps Pink' apples, where the weekly application of a NO dose between 5.3 and 5.9 μL L^{-1} kept the fruits greener after 7 days of shelf life [7]. However, in another study with 'Cripps Pink' apples, pre-storage treatments with NO application, regardless of the dose, the fruit did not differ from control (1-MCP) [9]. The results obtained in the present study and in the literature indicate that the effect of NO on maintaining skin background color can be variable among cultivars, as well as between different harvests.

NO acts as an anti-senescence agent, correlating negatively with the process in different plant species, as endogenous NO deficiency accelerates chlorophyll degradation [20]. The application of NO can delay chlorophyll breakdown by regulating the activity of chlorophyllase and Mg-dechelatase enzymes, reducing carotenoid accumulation, which is responsible for the yellow background color of apples, and by promoting the antioxidant system during ripening [19,21]. However, based on the skin background color data observed in this study, it is possible that the higher frequencies of NO application (at the beginning and end and at the beginning and every 30 days of storage) were detrimental or even cumulative, accelerating chlorophyll loss in the fruit, since the greener fruits were those treated only at the beginning of storage or not treated with NO. These findings suggest a negative impact of excessive NO application.

Changes in peel color indicate that frequent or excessive NO applications accelerated yellowing rather than preserving fruit appearance, which contradicts the expected senescence-delaying effect of NO. Zhu et al. [22], when treating 'Feicheng' peaches with 5, 10, and 15 μL L^{-1} of NO gas, observed toxicity from the highest dose of NO in the fruit, while 5 and 10 μL L^{-1} were beneficial. This contrary effect of NO, when in high doses, may be related to the formation of reactive nitrogen species (RNS) and reactive oxygen species (ROS), which can be harmful to metabolism [22,23]. In this case, NO, instead of inducing antioxidant activity, acts as a facilitator of cellular disorder [24]. 'Bartlett' pears treated with 50 and 10 μL L^{-1} of NO for 12 h showed accelerated ripening, unlike those treated with 10 μL L^{-1} of NO for 2 h, which showed a delay in this process [25], indicating that in addition to high doses, longer exposure periods can also have a negative effect. However, the mode of action of NO on chlorophyll degradation and/or carotenoid biosynthesis and accumulation in fruit still needs to be further investigated [21].

After 8 months in CA storage followed by 7 days on the shelf, no differences were observed for flesh firmness, SSC, and TA (Table 2). Similarly, no differences in flesh firmness were found with NO application for 'Cripps Pink', and 'Baigent' apples during storage [6,8]. However, 'Cripps Pink' apples stored in ultra-low oxygen (ULO-CA) for 8 months showed higher flesh firmness when treated weekly with NO at doses of 5.9 and 6.7 μL L^{-1} for less and more mature fruits, respectively [7]. The loss of flesh firmness is one of the main changes that occur during ripening, making fruits more susceptible to physical damage and fungal infections during handling. During ripening, several cell wall-modifying enzymes can cause structural modifications to pectin polymers, reducing cohesion between cells and leading to fruit softening [26]. Although no significant difference in flesh firmness were observed between control and NO-treated apples, all maintained their average values between 66 and 69 N, which is suitable for the commercialization of 'Fuji Mishima' apples [27]. This stability may indicate a positive effect of CA on firmness maintenance that overlapped the NO applications. The lower O_2 partial pressure during CA storage, may itself reduce various oxidative reactions such as respiration and ethylene biosynthesis, lowering the activity of cell wall breakdown enzymes responsible for softening [26].

Table 2. Flesh firmness, soluble solids content (SSC), titratable acidity (TA), flesh browning, and decay in 'Fuji Mishima' apples after 8 months of storage in a controlled atmosphere (1.0 kPa O_2 + < 0.5 kPa CO_2; 1.0 ± 0.2 °C; RH of 94 ± 2%) and 7 days of shelf life (20 ± 1 °C; RH of 65 ± 5%), as a function of nitric oxide (NO) application in two doses and at different times during storage. NO: nitric oxide.

Treatments		Flesh Firmness (N)	SSC (°Brix)	TA (% of Malic Acid)	Flesh Browning (%)	Decay (%)
NO Doses	Period of Application					
0 μL L^{-1}	No application	68.4 ^{ns}	15.6 ^{ns}	14.0 ^{ns}	14.0 ^{ns}	23.8 b
5 μL L^{-1}	Beginning	65.7	15.1	17.6	17.6	27.3 b
5 μL L^{-1}	Beginning and end	66.2	15.2	15.9	15.9	26.4 b
5 μL L^{-1}	Beginning + every 30 days	65.0	15.2	16.3	16.3	44.5 a
10 μL L^{-1}	Beginning	65.8	14.8	16.7	16.7	33.8 b
10 μL L^{-1}	Beginning and end	66.3	14.9	15.0	15.0	26.4 b
CV (%)		2.8	2.5	5.6	14.9	14.9

Means followed by the same letter in the column do not differ by the Scott–Knott test ($p < 0.05$). ns: not significant by ANOVA.

The SSC in all treatments was above 14.8 °Brix, which is also suitable for the fresh consumption of 'Fuji Mishima' apples [27] and was not influenced by the doses and frequencies of NO application. The same result was verified for 'Cripps Pink' apples, where SSC values were not affected by NO application, regardless of the maturity stage and duration of ULO-CA storage [7]. For 'Baigent' apples, the highest SSC values were observed for the treatment of 2 μL L^{-1} applied at the beginning, every 30 days, and at the end of the storage period, but not differing from control [6]. Under refrigerated storage, 'Cripps Pink' apples only showed differences in SSC between the control and the treatment with 20 μL L^{-1} NO applied for 2 h [8].

No differences were also observed regarding TA, with values remaining between 0.21 and 0.23% malic acid. In 'Cripps Pink' apples, the acid content was not affected by postharvest NO application [8]. On the other hand, in 'Baigent' apples, there was a decrease in TA with the use of higher NO doses, 10 μL L^{-1} at the beginning, and higher application frequencies, 5 μL L^{-1} every 30 days or at the beginning and end of the storage period [6]. For 'Cripps Pink' apples stored in ULO-CA, there was a linear increasing response, where 10 μL L^{-1} NO provided higher TA values for less mature apples, while for more mature apples, there was no difference between treatments [7]. This result highlights the importance of the effect of the maturity stage at harvest on the effect of NO on TA. The 'Fuji Mishima' apples used in this study were already mature at harvest, according to the quality parameters described by Fioravanço et al. [27] for the cultivar, which may have led to the lack of response to NO.

The treatments with NO also did not show an effect on flesh browning (Table 2). Temperature is the main environmental factor influencing the conservation of fruit postharvest. In general, apples are non-chilling sensitive fruits and can be stored at temperatures above the freezing point [21], yet they are susceptible to cold damage such as flesh browning. The postharvest application of NO can reduce cold injuries in fruit by decreasing membrane permeability, malondialdehyde content, lipid peroxidation, and ion leakage, effects associated with a reduction in ROS generation, ROS detoxification, and an increase in antioxidant enzyme activity or expression [28,29]. Flesh browning is attributed to the oxidation of phenolic compounds, while NO may inhibit the activity of enzymes such as phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD), thereby maintaining fruit quality [11]. For 'Baigent' apples, treatment with 5 μL L^{-1} NO at the beginning and end of storage in CA reduced the incidence of flesh browning [6].

The incidence of decay in 'Fuji Mishima' apples after 7 days of shelf life was highest in the treatment with application of 5 μL L^{-1} NO every 30 days of storage, while all other strategies of NO treatment did not differ from control (Table 2). For 'Baigent' apples, the application of NO, regardless of dose and application period, showed favorable results in reducing the incidence of decay [6]. NO has the potential to improve postharvest quality by acting in pest and disease control by inducing defense responses at the genetic and molecular level, depending on the dose and exposure time [5]. It also acts in the activation of enzymes related to defense metabolism, such as cinnamate 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), chalcone synthase (CHS), and chalcone isomerase, in addition to the accumulation of antifungal compounds, phenolic compounds, and antioxidant activity [11,21].

The NO application did not alter the phenolic compound content in the flesh or the antioxidant capacity quantified by the DPPH method (Table 3). The control and all frequencies of NO application at a dose of 5 μL L^{-1} showed fruits with higher phenolic compound content in the skin compared to treatments with 10 μL L^{-1} NO, indicating a possible negative effect of higher NO doses. On the other hand, the antioxidant capacity of the flesh, as measured by the ABTS method, was lower in fruits not treated with NO, while in the skin it was lower in untreated fruits, as well as in those treated with 5 μL L^{-1} NO only at the beginning and beginning + end of the storage period, and 10 μL L^{-1} NO applied at the beginning and end of CA storage. NO may end up inducing oxidative stress instead of suppressing it. In 'Micro-Tom' tomatoes, NO repressed the activity of H_2O_2 scavenging enzymes, contributing to increased ROS and RNS production [30].

Table 3. Total phenolic compound content and total antioxidant activity by ABTS and DPPH methods in the flesh and skin of 'Fuji Mishima' apples after 8 months of storage in a controlled atmosphere (1.0 kPa O_2 + < 0.5 kPa CO_2; 1.0 ± 0.2 °C; RH of 94 ± 2%) and after 7 days of shelf life (20 ± 1 °C; RH of 65 ± 5%), as a function of nitric oxide (NO) application in two doses and at different times during storage. TPC: total phenolic compound; GAE: gallic acid equivalent; TEAC: Trolox equivalent; DM: dry mass; FM: fresh mass; NO: nitric oxide.

Treatments		TPC Flesh	TPC Peel	ABTS Flesh	ABTS Peel	DPPH Flesh	DPPH Peel
NO Dose	**Period of Application**	**(mg GAE g^{-1}DM)**	**(mg GAE g^{-1}FM)**	**(mg TEAC g^{-1}DM)**	**(mg TEAC g^{-1}FM)**	**(% DPPH Inhibition)**	**(% DPPH Inhibition)**
0 μL L^{-1}	No application	2.14 ^{ns}	3.30 a	2.56 b	4.67 b	90.1 ^{ns}	84.3 ^{ns}
5 μL L^{-1}	Beginning	2.25	3.61 a	4.57 a	4.80 b	89.3	84.1
5 μL L^{-1}	Beginning and end	2.16	3.31 a	5.34 a	4.95 b	89.6	84.8
5 μL L^{-1}	Beginning + every 30 days	2.49	2.90 a	5.83 a	6.16 a	82.9	83.2
10 μL L^{-1}	Beginning	2.33	2.36 b	5.21 a	5.56 a	89.8	83.5
10 μL L^{-1}	Beginning and end	2.50	2.09 b	5.68 a	4.90 b	89.1	85.0
CV (%)		11.0	12.9	12.2	9.8	5.6	1.9

Means followed by the same letter in the column do not differ by the Scott–Knott test ($p < 0.05$). ns: not significant by ANOVA.

Gupta et al. [31] emphasize that NO has an ambiguous effect, acting as an antioxidant at low concentrations and as a pro-oxidant at high doses. In this context, the application of 5 μL L^{-1} NO, regardless of frequency, seems to have been more effective in increasing the phenolic compound content and antioxidant capacity in the skin and flesh of 'Fuji Mishima' apples. However, when 5 μL L^{-1} NO was applied every 30 days, it may have led to negative effects on fruit decay due to frequent exposure to the gas, again pointing to the negative effect of NO when applied in higher doses or frequencies. In parallel, these

fruits showed both higher phenolic compound content and antioxidant capacity, mainly in the skin, the primary barrier against pathogens. Frequent NO application may have caused structural damage to the fruit and compromised the barriers, leading to oxidative stress [21]. This contradictory effect, between higher decay incidence and the antioxidant system, may have been caused as a response to stress induced by frequent NO treatment, which led to an increase in decay occurrence. The metabolic response of the fruit to stress may have led to an increase in the synthesis of antioxidant metabolism compounds as a defense mechanism [31,32]. Despite minor variations in phenolic content and antioxidant activity, NO treatment every 30 days increased decay, indicating that higher application frequency may compromise fruit integrity during long-term storage.

It is well established in the literature that the effect of NO is dependent on the applied dose, exposure time, frequency of application during storage, and the fruit species used [6,8,21]. Although in the present study NO reduced ethylene production and suppressed the climacteric peak during the evaluation period, this did not result into delayed ripening and quality maintenance after storage. Other studies conducted with different cultivars, such as 'Baigent' and 'Cripps Pink', observed positive results of NO on ethylene production and fruit quality maintenance after storage [6,7]. This differentiated response among different cultivars raises the hypothesis that the effect of NO may not only be species-dependent but also cultivar-dependent. However, different clones of the 'Gala' cultivar also showed a differentiated effect to NO application during storage. As mentioned earlier, 'Baigent' apples showed positive results with NO application (5 μL L^{-1}) at the beginning and end of storage in CA (1.2 kPa O_2 + 2.0 kPa CO_2).

In 'Maxi Gala' apples, the application of NO (10 μL L^{-1}) at the beginning and end of storage did not suppress ethylene production in CA (1.2 kPa O_2 + 2.0 kPa CO_2) and increased phytohormone synthesis in a CA with extremely low O_2 partial pressure (pO_2 varying between 0.1 and 0.7 kPa) and under dynamic controlled-atmosphere conditions (minimum pO_2 of 0.08 kPa; maximum of 0.5 kPa; average of 0.18 kPa) [33]. Furthermore, the authors did not observe a positive effect of NO, regardless of storage conditions, on maintaining fruit quality. These results contrast with each other even when conducted with clones of apples from the same cultivar. Therefore, in addition to raising the hypothesis of a differential effect based on cultivar, it is possible that the effect of NO is dependent on storage conditions, maturity stage, and even the year of production. Future studies considering these aspects need to be conducted for a better understanding of the effects of NO during apple storage. The evaluation period of eight months corresponds to the long-term storage duration used commercially for Fuji group apples under controlled atmosphere conditions. This storage length was intentionally adopted to replicate realistic postharvest scenarios and to assess the persistence of NO effects over the actual marketing period. Shorter storage durations might reveal transient physiological changes, but they would not represent the fruit quality observed under commercial conditions.

Overall, the findings demonstrate that while NO influenced some metabolic processes, it was not effective in maintaining the quality parameters of 'Fuji Mishima' apples throughout prolonged controlled-atmosphere storage. These outcomes indicate that NO, under the tested conditions, does not extend storage duration or preserve marketable quality, reinforcing the cultivar's sensitivity and the need for complementary preservation methods.

4. Conclusions

The application of NO, in the evaluated doses and frequencies and under CA conditions of 1 kPa O_2 + < 0.5 kPa CO_2, 1.0 ± 0.2 °C, and 94 ± 2% RH, did not show a positive effect on maintaining the quality of 'Fuji Mishima' apples. The dose of 5 μL L^{-1} NO, applied every 30 days during storage, despite reducing ethylene synthesis, resulted in

more mature fruit and increased the incidence of decay in 'Fuji Mishima' apples. The results provide practical insight into the limited benefit of NO application for maintaining the postharvest quality of 'Fuji' apples and highlight the importance of adjusting storage strategies according to cultivar-specific responses. However, it is important to recognize that the effectiveness of NO may vary depending on cultivar characteristics, maturity stage at harvest, storage conditions, and gas concentration or exposure frequency. These factors can influence the metabolic and physiological responses of fruit to NO, which may explain the contrasting results reported among different studies and apple cultivars. Future research should focus on optimizing dose and application timing for diverse genotypes to clarify the mechanisms underlying these responses and enhance the practical use of NO in commercial storage systems.

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Avaliação de tolerância a baixas temperaturas em genótipos de arroz irrigado (*Oryza sativa* L.) na fase reprodutiva em experimento a campo, durante evento climático espontâneo na safra 2019/2020

Evaluation of low temperature tolerance in irrigated rice (Oryza sativa L.) genotypes at reproductive phase in a field experiment, during a spontaneous climatic event in the 2019/2020 crop season

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RESUMO

Aproveitando-se a ocorrência de um evento espontâneo de baixas temperaturas (<17 ºC) em experimento de campo de arroz irrigado, coincidente com a fase reprodutiva da cultura, foram avaliados a produtividade, a porcentagem de esterilidade de espiguetas e o número de panículas por metro quadrado. O experimento foi conduzido no sul de Santa Catarina, na safra 2019/2020, em delineamento de blocos ao acaso com três repetições no sistema pré-germinado. Do total de genótipos que compunham o experimento, 13 foram selecionados para comporem a análise principal, de esterilidade. Baseado numa série de estudos anteriores para estresse por baixas e altas temperaturas, a hipótese era de que a linhagem SC 806 se destacaria como resiliente. De fato, a SC 806 apresentou níveis de esterilidade significativamente menores que as testemunhas comerciais SCS116 Satoru, SCS122 Miura e SC 790 (SCS125), ao mesmo tempo que foi competitiva com as mesmas no tocante à produtividade. Assim, a SC 806 foi validada como resiliente a baixas temperaturas, confirmando o bom desempenho verificado em experimentos anteriores, e com isso demonstrando ser promissora como linhagem candidata à lançamento na forma de variedade.

PALAVRAS-CHAVES: esterilidade; frio; antese; microsporogênese; mudanças climáticas; resiliência

ABSTRACT

Concerning the occurrence of a spontaneous event of low temperatures (<17 ºC), in an irrigated rice field experiment, during the reproductive phase of the crop, yield was evaluated as well as percentage of grain sterility and number of panicles. The experiment was conducted in the south of Santa Catarina, in the 2019/2020 crop season, having been water-seeded in randomized blocks with three replications. Of the total number of genotypes that made up the experiment, 13 were selected to make up the main analysis, of sterility. Based on a series of previous studies for stress due to low and high temperatures, the hypothesis was that the SC 806 line would stand out as resilient. Based on a series of previous studies for low and high temperature stress, the hypothesis was that the SC 806 inbreed line would stand out as resilient. In fact, SC 806 showed significantly lower sterility levels than the commercial controls, SCS116 Satoru, SCS122 Miura and SC 790 (SCS125), while remaining competitive in terms of yield. Thus, SC 806 confirmed its resilience to low temperatures, what was a behavior showed in previous experiments, proving to be promising as a candidate for release as cultivar soon.

KEY-WORDS: sterility; cold; anthesis; microsporogenesis; climate change; resilience

As mudanças climáticas promovem o aumento na frequência de extremos de temperaturas, e estas usualmente causam redução na biomassa e produtividade vegetal, o que coloca a humanidade num sério dilema, em virtude de se estar diante do desafio de, no mínimo, dobrar a produção de alimentos até 2050 (LIU 2013). A vulnerabilidade da orizicultura do Sul do Brasil a eventos extremos de temperaturas coloca em risco a segurança alimentar do país. As baixas temperaturas (NAJEEB et al. 2021), bem como as demasiado altas (YE et al. 2021), provocam esterilidade no arroz, diminuindo a produtividade e consequentemente baixam a produção total deste cereal, além de diminuírem a qualidade do produto final. Neste sentido, o melhoramento genético talvez seja a melhor estratégia para que a agricultura brasileira enfrente e conviva com as alterações climáticas (RAMALHO et al. 2009).

As baixas temperaturas afetam particularmente o arroz em Santa Catarina no verão, em períodos nos quais a cultura frequentemente está em fase reprodutiva. Quando frentes frias invadem o Estado, especialmente as regiões orizícolas de elevada altitude são particularmente expostas a temperaturas menores do que no restante do Estado. Há estudos apontando perdas por frio variando de 80% no norte do Japão, 26% na Coréia do Sul anualmente, enquanto na Austrália perde-se anualmente de 0,5 a 2,0 t ha^{-1}. No nordeste da China, as perdas por frio ocorrem uma vez a cada 3 ou 5 anos, causando perdas de produtividade de 30% a 60% (XIAO et al. 2021). No Brasil, considera-se que temperaturas entre 15-17º C induzem à esterilidade em variedades tolerantes de arroz irrigado, e de 17 a 19º C em variedades sensíveis (SOSBAI, 2018), sendo a fase mais crítica a microsporogênese (R2) e a antese (R4), sendo a primeira usualmente considerada a mais sensível delas (ROZZETTO et al. 2015, SOUZA et al. 2017, XIAO et al. 2021, MARSCHALEK et al. 2019b).

Diferentes estratégias podem ser usadas para aumentar a tolerância do arroz a extremos de temperatura, e uma delas ainda é a seleção fenotípica (KHAN et al. 2019). Entretanto, a temperatura é um fator imprevisível e difícil de utilizar como agente seletivo em programas de melhoramento. Apesar disso, progressos têm sido alcançados na obtenção de cultivares resistentes ao frio, como em Hokkaido, no Japão (SHINADA et al. 2013) e também em sítios de altitude nas regiões tropicais do Kenya a seleção para tolerância a frio em estágios reprodutivos em experimentos de campo sob condições naturais foi descrito como bem-sucedido, confirmando o comportamento prévio dos genótipos avaliados anteriormente sob condições controladas (SAMEJIMA et al. 2020). Há resultados que indicam avanços similares no programa de melhoramento genético da Epagri (Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina), já que estudos apontam que há considerável variabilidade para este caráter nas linhagens de arroz desenvolvidas pela instituição (SOUZA et al. 2017, STÜRMER et al. 2017, STÜRMER et al. 2019, MARSCHALEK et al. 2019b). Também o Instituto Rio Grandense do Arroz (IRGA) logrou êxito na seleção, lançando a cultivar de arroz IRGA 426, bem como a Embrapa com a cultivar BRS Querência, tolerantes a frio na fase de plântula (SOSBAI 2018)

Deste modo, repousa no melhoramento genético, a esperança de que a orizicultura sul-brasileira possa conviver com as mudanças climáticas através do desenvolvimento e adoção de variedades que tolerem temperaturas extremas. Assim sendo, o presente estudo procurou aproveitar um destes eventos naturais espontâneos de baixas temperaturas, ocorrido no âmbito de um experimento VCU (Valor de Cultivo e Uso), para averiguar os diferentes graus de esterilidade das linhagens de arroz irrigado expostas a este estresse na fase reprodutiva. Especial interesse havia no comportamento de uma dada linhagem, considerada por vários estudos prévios como tolerante às baixas temperaturas na fase reprodutiva. A hipótese era, portanto, a de que um dos genótipos, a linhagem SC 806, evidenciasse o mesmo comportamento promissor já obtido em condições controladas de estresse (STÜRMER et al. 2017, STÜRMER et al. 2019), e confirmasse também o mesmo bom desempenho, obtido anteriormente em avaliações de campo em experimentos durante cinco safras agrícolas em regiões de altitude (500 a 600m) de Santa Catarina (MARSCHALEK et al. 2015, 2017, 2019a).

O experimento foi conduzido na safra 2019/2020 no Centro de Treinamento da Epagri de Araranguá (CETRAR). A semeadura (24/10/2019) deu-se em parcelas de 3 x 4m, com três repetições por genótipo, em blocos ao acaso, usando 120 kg ha^{-1} de sementes, e a condução do experimento seguiu as recomendações da Epagri (EBERHARDT et al. 2015), mas sem o uso de fungicidas. Apesar do VCU ser composto, naquela safra, por 23 genótipos, apenas 13 deles foram escolhidos para fazer parte das estimativas de esterilidade, por representarem grupos contrastantes ou ainda em avaliação quanto à tolerância ao frio.

Em fevereiro de 2020 houve então eventos de baixas temperaturas, sendo que nas noites de 11 e 14 de fevereiro as temperaturas mínimas noturnas ficaram por algumas horas em torno dos limítrofes 17 ºC. Neste período, as linhagens de ciclo médio e médio-tardio estavam em plena antese (R4) (COUNCE et al. 2000). Tais linhagens estiveram também expostas a frio durante a microsporogênese (R2), quando, as

temperaturas mínimas noturnas variaram de 15 ºC (dia 17/01/20) a 17,5 ºC (25 e 26/01/20). Já durante as noites de 22 a 24 de fevereiro (três dias), e de 27 de fevereiro à 1º de março (quatro dias), as temperaturas mínimas noturnas caíram abaixo dos 17 ºC, variando de 14,3 ºC até 16,9 ºC, período no qual, desta vez, eram as linhagens e cultivares tardias que estavam em antese (R4). Estas linhagens e cultivares tardias tiveram seu período de microsporogênese (R2) aproximadamente de 11 a 14 de fevereiro, período que, como relatado anteriormente, também foi de baixas temperaturas. Assim, ainda que de forma não uniforme e exata, todos os genótipos do experimento estiveram expostos a baixas temperaturas noturnas durante o período reprodutivo crítico, tanto na microsporogênese quanto nos dias em que estava ocorrendo antese.

Diante disso, e antevendo-se uma inevitável esterilidade decorrente dos referidos eventos de baixas temperaturas, resolveu-se aproveitar a oportunidade e o transcorrer do experimento VCU, selecionando 13 genótipos para uma amostragem, visando estimar o índice de esterilidade das linhagens. Os 13 genótipos comporiam, com base em estudos anteriores, dois principais grupos extremos: (1) linhagens que apresentaram perspectiva de tolerância a baixas temperaturas (SC 911, SC 775, SC 806); (2) cultivares comerciais e linhagens, sensíveis ao frio na fase reprodutiva (SC 1029, SCS116 Satoru, SCS122 Miura, SC 790), além de alguns outros genótipos cujo comportamento se desejava avaliar (MARSCHALEK et al. 2015, ROZZETTO et al. 2015, MARSCHALEK et al. 2017, STÜRMER et al. 2017, SOUZA et al. 2017, MARSCHALEK et al. 2019a,b).

A colheita de amostras para avaliação da esterilidade e contagem de panículas deu-se em amostras de 0,5 x 0,5 m, sendo que estas tiveram as espiguetas debulhadas manualmente, a fim de preservar unidas a pálea e a lema (casca) das espiguetas estéreis. A amostra total de cada parcela foi quarteada, e as espiguetas férteis (grãos cheios) foram separadas das espiguetas vazias através de um soprador colunar, com fluxo de ar ascendente, e todas foram contadas (Contador, Pfeuffer, Alemanha). Foram determinadas para cada amostra (parcela): número de panículas e esterilidade, sendo que da última se infere a tolerância à frio, comparando-se o grau de esterilidade de diversos genótipos com as principais testemunhas cultivadas, e associando este resultado com dados de outros estudos. Também efetuou-se a avaliação da produtividade por meio da colheita de uma área de 2 x 2m, sendo a mesma obtida através do peso corrigido da parcela para 13% de umidade dos grãos após estimação da umidade da amostra (medidor de umidade de grãos Gehaka G800).

A análise de variância foi feita utilizando-se o software Assistat versão 7.7 (SILVA & AZEVEDO 2016). Os valores do teste F foram considerados significativos a 5% ($P<0.05$) e muito significativos a 1% ($P<0.01$). Quando significativos, efetuou-se teste de comparação múltipla de médias de Duncan em nível de 5% de significância.

A análise de variância para número de panículas por metro quadrado e esterilidade revelaram diferenças significativas ($P<0.05$) entre os genótipos, assim como a produtividade ($P<0.01$), mas estes três parâmetros não revelaram diferenças entre blocos, o que atesta a uniformidade da área experimental e a qualidade do experimento e das avaliações. Os coeficientes de variação (Tabela 1) do experimento mantiveram-se em patamares que denotam confiabilidade. A Tabela 1 mostra os resultados dos testes de médias, com os genótipos ordenados da maior para a menor esterilidade.

Dados desta natureza, obtidos de experimentos de campo, no qual o estresse térmico não pode ser controlado, poderiam ser encarados com ceticismo, já que as linhagens que compõem um VCU não são todas do mesmo ciclo. O diferente ciclo dos genótipos avaliados poderia ensejar incidência do estresse em diferentes estádios morfo-fisiológicos. Ou seja, poderia se levantar a suspeita de que diferentes níveis de esterilidade pudessem ser resultado de diferentes graus de escape dos genótipos. Entretanto, os dados meteorológicos comprovam a atuação de massas de ar frio durante o experimento por um período considerável de dias, atingindo assim os diferentes genótipos. Além disso, para que se descarte a suposição de escape do frio em virtude do ciclo dos 13 genótipos avaliados, entre os quais havia os de ciclo tardio, e os de ciclo médio, basta verificar os dados (Tabela 1). Constata-se que o ciclo dos genótipos foi aumentado pela condição climática (frio) e tanto os genótipos de ciclo mais curto, quanto os de ciclo mais longo, tiveram sua esterilidade afetada pelo estresse térmico (Tabela 1), o que inclusive era de fácil constatação visual (Figura 1). Assim, é inequívoco que também a linhagem SC 806, alvo da hipótese de tolerância, não tenha sido poupada do estresse, portanto, seu melhor desempenho não tem por causa eventual escape. Corroborando estes dados, também em evento espontâneo de baixas temperaturas na antese na safra 2020/2021 (unidade de observação, Taió, SC), a SC 806 apresentou menor esterilidade e maior produtividade do que as testemunhas (MARSCHALEK et al. 2022).

Tabela 1. Panículas m^{-2}, esterilidade (%) e produtividade (kg ha^{-1}) em 13 linhagens e variedades de arroz irrigado (medias de três repetições), em experimento VCU de Araranguá, na safra 2019/2020.

Table 1. Panicles m^{-2}, sterility (%) and yield ((kg ha^{-1}) in 13 irrigated rice inbreed lines and varieties (means of three repetitions), in a VCU field experiment in Araranguá, in the 2019/2020 crop season.

Genótipo	Panículas m^{-2}		Esterilidade (%)		Produtividade (kg ha^{-1})		Ciclo
SC 1029	851	bcd	41,0	a	7817	bc	171
SC 790	941	abcd	36,0	ab	8836	bc	165
SCS116 Satoru	920	bcd	33,2	abc	7952	bc	163
SCS122 Miura	724	d	30,7	abc	9282	bc	164
BRS A704	1083	abc	29,4	abc	8139	bc	152
SC 908	1261	a	29,3	abc	8785	bc	166
SC 971	1057	abcd	28,5	abcd	6548	c	139
SCS124 Sardo	889	bcd	28,1	abcd	7383	bc	149
SC 911	883	bcd	25,6	abcd	10099	b	167
SC 1026	800	cd	23,7	bcd	10213	b	168
SC 914	865	bcd	22,8	bcd	7650	bc	172
SC 775	913	bcd	17,6	cd	12977	a	160
SC 806	1179	ab	13,3	d	9897	b	150
CV(%)	18,4		29,9		16,6		

*Médias seguidas pela mesma letra (coluna) não diferem estatisticamente pelo teste Duncan ao nível de 5% de significância.

Figura 1. Constatação visual da menor esterilidade da linhagem SC 806 (centro) em comparação com as variedades SCS122 Miura (à esquerda, e mais tardia que a SC 806) e SCS124 Sardo (a direita e mais precoce), evidenciando a impossibilidade de que a SC 806 tenha sido favorecida pelo escape ao estresse térmico por baixa temperatura.

Figure 1. Visual observation of the lower sterility of the SC 806 inbreed line (center) in comparison with the varieties SCS122 Miura (on the left; a few days later-maturing cultivar compared with the inbreed line SC 806) and SCS124 Sardo (on the right, an earlier-maturing cultivar, compared with SC 806), demonstrating the impossibility that the SC 806 has been favored by escape from low temperature stress.

Percebe-se ainda o grande número de perfilhos férteis (panículas) da SC 806 (Tabela 1), resultantes do excelente perfilhamento. Também fica evidente a classificação desta linhagem como a menos estéril do grupo, o que foi de fácil constatação visual no pós-colheita (Figura 1). Admite-se, entretanto, que os valores observados de esterilidade possam eventualmente ter, embutidos, outras causas, que não apenas o estresse por frio, pois os VCUs de arroz da Epagri são conduzidos sem fungicidas, logo, patógenos também poderiam ter causado parte da esterilidade observada. Entretanto, se a brusone, principal doença do arroz, tivesse desempenhado papel preponderante na esterilidade observada nos genótipos, os resultados seriam favoráveis à genótipos como a linhagem SC 790 (lançada em 2020 como cultivar SCS125) e a cultivar SCS122 Miura, que são considerados, desde seu lançamento, como médio-resistentes, portanto, baixos índices de esterilidade seriam esperados nestes dois genótipos, enquanto seriam agravados em outros. Entretanto, o que se deu foi o contrário.

O fato a se ressaltar aqui, é que variedades estabelecidas na orizicultura catarinense, referências em produtividade, como a SCS116 Satoru e a SCS122 Miura, apresentaram esterilidades maiores que a SC 806, que também as superou em produtividade no experimento aqui relatado. Eliminadas as hipóteses de bom desempenho da SC 806 devido à escape e fitopatógenos, a rigor, poder-se-ia ainda contestar as diferenças encontradas neste estudo, posto que não foram estatisticamente significativas para produtividade no agrupamento “b” (Tabela 1) de médias (Duncan 5%). Entretanto, cabe lembrar que em melhoramento de cereais o ganho genético de produtividade é abaixo de 1% ao ano, logo, detectar diferenças estatisticamente significantes num grupo de genótipos elite é tarefa cada vez mais difícil. Além disso, mesmo que as aludidas diferenças não sejam relevantes estatisticamente, isso não significa que sejam desprezíveis econômica e geneticamente. A tomada de decisão sobre os lançamentos de novas variedades não raro se dá através da análise de muitos outros atributos de uma linhagem candidata. Isso justifica o olhar atento que se coloca sobre a baixa esterilidade da SC 806 em relação a outros genótipos, o que já havia sido foi observado em dois estudos da Epagri (STÜRMER et al. 2019, FACCHINELLO et al. 2022), nos quais reporta-se esterilidades estatisticamente menores na SC 806 em experimentos de estresse térmico por frio na microsporogênese conduzidos em ambientes de temperatura controlada. Além disso, essa resiliência da SC 806 a baixas temperaturas, verificada pelos dados deste evento espontâneo ora relatado, é corroborada por resultados de cinco experimentos em diferentes safras em locais de elevada altitude, sujeitos à menores temperaturas, que demonstraram que a SC 806 apresenta boas produtividades, similares ou superiores às testemunhas SCS116 Satoru e SCS122 Miura (MARSCHALEK et al. 2015, 2017, 2019a,b). Todas estas evidências, validam-se mutuamente, e confirmam que os dados do estudo ora apresentado, não são obra do mero acaso ou de escape, mas sim, indicam uma consistente maior resiliência da linhagem SC 806, que mantém desempenho coerente em experimentos ao longo de vários anos, desde ensaios de campo em locais de altitude, até em experimentos com temperatura controlada.

Outra particularidade da SC 806, descoberta durante trabalhos colaborativos entre UDESC e Epagri (tese de doutorado), foi sua tolerância a altas temperaturas (SOUZA et al. 2022). Isso pode indicar a existência de mecanismos sobrepostos de tolerância no período reprodutivo, o que já foi reportado por XIONG (2013). As altas temperaturas também ocorrem frequentemente e afligem a produção de arroz de Santa Catarina, sendo um destes eventos registrado por EBERHARDT (2010).

Além da SC 806, outra linhagem que apresentou bom desempenho foi a SC 775, todavia, esta não foi considerada promissora, e não mereceu destaque no presente texto, porque apesar de ter apresentado baixa esterilidade e alta produtividade (Tabela 1), o bom desempenho da mesma não se confirmou na média dos quatro anos em que esteve em análise em 5 VCUs conduzidos anualmente em Santa Catarina (dados não apresentados). Além disso, apresentou desempenho sensorial inferior, sendo descartada após a safra 2019/2020. Já a SC 806 foi avaliada agronomicamente durante cinco anos nos VCUs, e apresentou bom desempenho agronômico, sensorial e de qualidade de grãos. Portanto, os resultados de esterilidade e produtividade da SC 806, a tornam uma candidata a ser lançada como cultivar comercial em breve (sementes comerciais estão em produção na safra 2022/2023), estando inclusive já registrada e protegida como variedade no RNC e SNPC (MAPA).

Concluindo, houve evidentes diferenças entre os genótipos avaliados, principalmente quanto ao nível de esterilidade que acometeu cada um deles diante do estresse térmico por baixa temperatura. A linhagem de arroz irrigado SC 806, considerada resiliente à baixas temperaturas na fase reprodutiva por diversas pesquisas anteriores, e validada neste estudo de caso, uma vez lançada como variedade comercial, e adotada pelos produtores, pode ajudar a mitigar e tamponar os eventuais riscos à produtividade decorrentes das mudanças climáticas em curso, propiciando maior segurança alimentar.

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CULTIVAR RELEASE

SCSBRS126 Dueto: irrigated rice variety resilient to cold and heat at reproductive stage

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Abstract: *SCSBRS126 Dueto is a late-maturing high yield performing cultivar released by Epagri/Embrapa/Udesc. It has high tillering capacity, but poor resistance to lodging, medium resistance to blast, with long, slender grains and good sensory/cooking traits, and it has lower sterility under cold stress at microsporogenesis/anthesis and under heat at anthesis.*

Keywords: Oryza sativa*, Extreme temperatures, Low and high temperatures, climatic changes*

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INTRODUCTION

Santa Catarina State is the second largest irrigated rice producer in Brazil, with a cultivated area of 148.290 ha (2020/2021 crop season), which represents 10.6% of Brazilian rice area (Padrão 2022). Grown in 93 municipalities in Santa Catarina, rice is concentrated on the South Coast (61.9%), followed by the Middle/Lower Itajaí Valley and North Coast region (25.2%), Upper Itajaí Valley (9.04%) and Coast Center (3.9%) (Padrão 2022). From this total area, 3.66% (5.476 ha) are above 400 m altitude, and this number reaches 8.08% (12.095 ha) if a limit above 300 m is considered. On the other hand, 78.05% of Santa Caratina´s rice fields are cultivated at altitudes from 0 to 50 m, and 11% below the 50 to 100 m range, with 2.63% from 100 to 150 m. There is no cultivation between 150 and 300 m. Altitude plays an important role in temperature, which is decisive for rice, and therefore it is an important point, since climate change is affecting temperatures.

Climate change increases the frequency of extreme temperatures, and these usually lead to yield reductions, which puts humanity in a serious dilemma, due to the challenge of at least doubling the production of food by 2050 (Liu 2013). The vulnerability of rice farming in southern Brazil to extreme temperature events puts the country's food security at risk. Low temperatures (Najeeb et al. 2021), as well as those that are too high (Ye et al. 2021), cause

sterility in rice, decreasing yield and consequently lowering the total production and final quality of this cereal.

Low temperatures particularly affect rice in Santa Catarina especially in periods when the rice plant is in the reproductive stage. When cold masses invade the state, especially the high altitude regions are at risk. There are studies indicating annual losses in rice production due to cold reaching 60-80% (Xiao and Chen 2021). In Brazil, temperatures between 15 and 19 ºC are considered to induce sterility in irrigated rice varieties (Sosbai 2018), with the most critical phase being microsporogenesis (R2) and anthesis (R4), the first one usually being considered the most sensitive of them (Rozzetto et al. 2015, Souza et al. 2017, Xiao and Chen 2021). Concerning the high temperatures, they reduce anther dehiscence and pollen fertility rates. Some data estimate that a 1 °C increase in the temperature would decrease rice yield by 10%, and rice can be affected at microsporogenesis and anthesis. Temperatures above 35 °C at the flowering stage are considered to be at a critical level that harms floret fertility and grain yield, which occasionally could cause up to 80% loss (Buu et al. 2021).

Different strategies can be used to increase rice tolerance to extreme temperatures, and one of them is phenotypic selection (Khan et al. 2019), despite the temperature being an unpredictable factor and therefore difficult to use as a selective agent in breeding programs. Nevertheless, progress has been achieved in obtaining cold resistant cultivars; for instance, in Hokkaido, Japan (Shinada et al. 2013) and also in high altitude sites in tropical regions of Kenya, selection for cold tolerance in reproductive stages in field experiments was described as successful (Samejima et al. 2020). There are results that indicate progresses, in a similar way, in the breeding program of Epagri (Santa Catarina State Agricultural Research and Rural Extension Agency) (Rozzetto et al. 2017, Souza et al. 2017, Stürmer et al. 2019).

Despite all the efforts and resources spent on gene identification for cold and heat tolerance, the use of marker assisted selection for cold tolerance in breeding has crucial barriers, related to the lack of consistency of the quantitative trait loci (QTLs) governing this trait for any given growth stage over different genetic backgrounds under different low-temperature stress conditions (Xiao and Chen 2021, Lv et al. 2019, Najeeb et al. 2021, Ye et al. 2021). Besides that, molecular mechanisms related to heat stress remain unknown, so some promising molecular technologies seem not to be able to deliver proper solutions, like the transgenic efforts for heat tolerance, which have failed to release any commercial cultivars in the last 20 years (Buu et al. 2021).

Therefore, still relies on conventional breeding the hope that we can cope with the challenges of climate change developing varieties that tolerate extreme temperatures (Ramalho et al. 2009). In this context, Epagri´s Rice Team, concerned with issues related to climate change, began in 2008 studies with tolerance to low temperatures in the reproductive stages, which was a demand from rice farmers of the High Itajaí Valley region.

PEDIGREE AND BREEDING METHOD

The SCSBRS126 Dueto (SC 806 inbreed line) is the 26th rice cultivar released by Epagri's Irrigated Rice Breeding Program for Santa Catarina, in partnership with Embrapa, with support of Udesc/CAV. The name "Dueto" alludes to the inter-institutional partnership between Epagri and Embrapa, in addition to referring to the dual aptitude of the new variety, which is tolerant to low and high temperatures in the reproductive stage. Its release is dedicated to Dr. Orlando Peixoto de Morais (in memoriam), from Embrapa Rice and Beans, and to M.Sc. Richard Elias Bacha, former scientist at Epagri. The new variety results from the cross between the IRGA 424 variety and the Embrapa line BRA040081 (BRS Pampa variety), which was carried out in 2008 by Embrapa Rice and Beans. The F1 was carried out in 2009 (Goianira/GO), at the Palmital Experimental Station (EEP) and the F2 generation was conducted at Epagri – Itajaí Experimental Station (EEI) (2009/10). In 2010/11 the F2:3 families were evaluated in the Family Observation Test (EOF) at the EEP and in the "F2:3 Field Trial" at the EEI, with five families being selected, which were advanced to the F2:4 Family Yield Test (ERF) and evaluated in the 2011/12 crop season in Alegrete (RS), Capão do Leão (RS), Santa Vitória do Palmar (RS), and Itajaí (SC), where the family CNAx16810-B-7-B stood out. The genotype was then kept under study only at Epagri, which took this family to the F5 level (2012/13), and later to F6 and F7 (2013/14 and 2014/15) at EEI, as SC 806 inbred line.

Posteriorly, supported also by phenotypic selection field trials in autumn-winter at the EEI, SC 806 was standing out as an interesting genotype, and after that was included in normal spring-summer crop season experiments at 600 m (Rio do Campo/SC, 2014/15 to 2015/16) and 500 m altitude (Mirim Doce/SC, 2016/17). The SC 806 line showed good performances at these high altitude sites, and it was advanced to the official Value for Cultivation and Use trials (VCU)

in 2017/18.

At the same time (2015 - 2020), experiments were carried out at the EEI in partnership with the Agroveterinary Center of the State University of Santa Catarina – Udesc/CAV, in a controlled growth chamber (Instalafrio; software Sitrad). These studies confirmed the resilience of SC 806 not only to low temperatures (15-17 ºC) but also to high temperatures (up to 38 ºC), which can be summarized by a lower sterility percentage when compared to other cultivars (Stürmer et al. 2019, Souza et al. 2022). However, SCSBRS126 Dueto only has intermediate cold tolerance in germination and vegetative stage.

TRAITS PERFORMANCE

The agronomic/morphological traits of the SCSBRS126 Dueto variety are given in Table 1. SCSBRS126 Dueto has a late maturing cycle in Santa Catarina (SC) (142-144 days), excellent tillering at low altitudes, modern type of plant, and upright, hairy but short leaves. It is susceptible to lodging in pre-germinated system at low altitudes (SC at ≤ 400m), and moderate susceptible at higher altitudes (≥ 400m), whereas no lodging was observed in direct seeding system in Rio Grande do Sul State (RS). The variety also has low shattering and moderate susceptibility to blast (*Pyricularia oryzae*),

Table 1. Morphological and agronomic traits of SCSBRS126 Dueto in VCU[3]/DHS[4] trials

Plant trait / descriptor		**Description or Code (MAPA[1] - IRRI[2])**
Leaf color	[1, 4]	Green (2)
Leaf pubescence	[1, 4]	Medium (3)
Auricle color	[1, 4]	Green (1)
Ligule color	[1, 4]	Green or colorless (1)
Flag leaf angle	[1, 4]	Upright (1)
Plant height (culm length)	[1, 3, 4]	87 cm at the DHE Trials (76 cm at VCU)
Culm width	[1, 4]	4.1 mm
Tillering angle	[1, 4]	Upright (1)
Internode color	[1, 4]	Light Green (1)
Anthocyanin node color	[1, 4]	Absent/Weak (1)
Panicle length	[1, 4]	25.7 cm
Type of panicle	[1, 4]	Intermediate (3)
Panicle exsertion	[1, 4]	Complete (1)
Shattering	[1, 4]	Intermediate (3)
Awns	[1, 4]	Present (0), but rare
Awns length	[1, 4]	------- (1) (≈ 1-2 mm)
Stigma color	[1, 4]	Green (1)
Lemma/Palea pubescence	[1, 4]	Medium (5)
Keel's color (flowering)	[1, 4]	Green (2)
Keel's color (maturing)	[1, 4]	Yellow (2)
Glume color (palea/lemma)	[1, 4]	Golden/straw (1)
Sterile lemmas color (glumes)	[1, 4]	Straw (1)
Cycle to maturity	[1, 3]	Late maturing (142 -144 Days)
Leaf blast	[1, 3]	Moderately resistant
Panicle blast	[1, 3]	Moderately resistant
Brown spot	[1, 3]	Moderately susceptible
Leaf Scald	[1, 3]	Moderately susceptible
Sheath rot	[1, 3]	Moderately susceptible
False smut	[1, 3]	Moderately susceptible
Iron toxicity tolerance	[2]	Moderately susceptible (5.7)
Tillering	[1, 3]	Very High
Lodging (≥ 400 m altitude)	[1, 3]	Moderately susceptible
Lodging (≤ 400 m altitude)	[1, 3]	Susceptible

[1] MAPA (Ministry of Agriculture, Livestock and Food Supply) (2008) Descritores mínimos de arroz; [2] SES/IRRI (Standard Evaluation System) (IRRI 2013) www.clrri.org/ver2/uploads/SES_5th_edition.pdf>; [3] VCU trials (Itajaí, Massaranduba, Mirim Doce, Turvo, in 2017/18 and 2019/20); [4] DHS - distinguishability, homogeneity, stability (MAPA) evaluations 2018/19 to 2020/21

brown spot (*Bipolaris oryzae*), false smut (*Ustilaginoidea virens*) and sheath rot (*Sarocladium oryzae*).

The SCSBRS126 Dueto showed an average yield of 10,290 kg ha^{-1} in the pre-germinated VCU trials in Santa Catarina during tree crop seasons, which is a competitive yield compared to Epagri´s best control cultivars (Table 2). The same good performance was reached by Embrapa in the VCU trials in Rio Grande do Sul under direct seeding system in dry soil (Table 2).

SCSBRS126 Dueto has long, slender translucent grains, with lower percentage of chalked grains and chalked grain area, with good milling quality for white milled rice (Table 3), which are desirable traits to become accepted for white milled as well as for parboiled rice. All the physical grain analyses were carried out with 100g rough rice samples dehulled and milled by the Testing Rice Mill Device Suzuki (Model MT.08 - Suzuki, Santa Cruz do Rio Pardo/SP) and then analyzed by the Image Rice Grain Scanner (Selgron, Blumenau/SC) at the Epagri's Rice Breeding Laboratory (Lamgen/EEI). The sensorial and cooking traits of SCSBRS126 Dueto are comparable or better than those of the control cultivars, since the grains show soft texture, good aroma and taste (Table 4) (Quality Laboratory of Urbano Agroindustrial - Jaraguá do Sul/SC, Brazil).

Table 2. Average grain yield (kg ha^{-1}) of SCSBRS126 Dueto in Santa Catarina compared to control cultivars, in 12 VCU trials: 4 locations, in three crop seasons (2017/2018 to 2019/2020) under pre-germinated system; and average grain yield (kg ha^{-1}) of SCSBRS126 Dueto in Rio Grande do Sul (RS) compared to control cultivars, in VCU trials at four locations, in two crop seasons (2020/2021 to 2021/2022) under direct seeding system in dry soil

Location SC	**Itajaí**			**Massaranduba**			**Mirim Doce**			**Turvo**			**Average[1]**
Crop season	**17-18**	**18-19**	**19-20**	**17-18**	**18-19**	**19-20**	**17-18**	**18-19**	**19-20**	**17-18**	**18-19**	**19-20**	
Cultivar													
SCSBRS126 Dueto	10.788	9560*	13.696	9.731	9.405	12.536	10.586	9.733	7.695	10.708	11.229	7820**	10.290a
SCS116 Satoru	10.673	10.334	11.975	7.358	8.504	11.194	7.689	9.160	8.839	10.625	7.197	9.347	9.408a
SCS122 Miura	12.265	11.233	14.175	8.543	8.332	10.337	8.779	10.037	9.241	10.042	7.918	8.909	9.984a

Location RS	**Alegrete**				**Uruguaiana**					**Capão do Leão**		**Sta Vitória do Palmar**	**Average[2]**
Crop season	**20-21**		**21-22**		**20-21**		**21-22**			**20-21**	**21-22**	**21-22**	
Cultivar	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial1	Trial 2		Trial1	Trial 2	Trial 1	
SCSBRS126 Dueto	12.281	12.200	13.362	13.394	14.422	13.588	14.059	7.170	**	11.698	10.749	7.126	11667a
BRS Pampeira	11.284	11.394	13.226	12.809	15.703	12.238	13.761	8.718	**	10.696	10.223	7.582	11386a
BR-IRGA 409	10.700	10.556	9.899	10.295	10.803	9.984	11.211	12.827		9.716	8.898	6.624	9907b

[1, 2] Means with different letters are significantly different by Scott-Knott test at 1% probability (ANAVA by software GENES/UFV with [1] CV 8.5 % [2] CV 9.38 %). * Lodging; ** Underestimated due to birds attack

Table 3. Grain traits of SCSBRS126 Dueto milled rice grains at 3 years VCU's (2017/18 to 2019/20): Itajaí, Massaranduba, Mirim Doce and Turvo (SC – Brazil)

Trait	**Cultivar**		
	SCSBRS126 Dueto	**SCS116 Satoru**	**SCS122 Miura**
Total milled grains (%)[3]	67.94	66.10	66.95
Whole grains (%)[3]	60.01	56.72	58.65
Broken grains (%)[3]	7.93	9.38	8.30
Amylose content (%)[1]	24.28	24.87	25.00
Gelatinization temp.[1]	6.98	2.55	2.58
Grains chalked area (%)[3]	4.24	4.02	2.81
Chalked grains (%)[3]	6.66	6.95	5.08
Grain length (mm)[3]	7.09	7.13	7.24
Grain width (mm)[3]	1.99	2.16	2.09
Grain thickness (mm)[3]	1.73	1.72	1.70
Length-Width ratio[3]	3.56	3.30	3.46
Thousand grain weight[4]	27.75	28.5	27.9

[1] Embrapa/CNPAF (International Standard Method ISO 6647 2007); [2] Martínez C and Cuevas F (1989); [3] Analyzed with "Image" Scanner (Selgon) at LAMGEN (Rice Breeding Laboratory - Epagri) (Marschalek et al. 2017). [4] Analyzed at LAMGEN (Rice Breeding Laboratory - Epagri)

Table 4. Means of SCSBRS126 Dueto sensory traits of the grains (2017/2018 to 2019/2020)

	Texture	Color	Stickiness	Characteristic odor	Characteristic taste
SCSBRS126 Dueto	2.0	2.4	2.9	0.5	0.8
SCS116 Satoru	3.4	1.3	5.2	1.0	0.9
SCS122 Miura	3.2	1.8	5.2	0.8	0.9

ABNT (1998) NBR14140 sensory analysis - methodology - Quantitative descriptive analysis (QDA) - Food - Beverage. Available at https://www.abntcatalogo.com.br/norma.aspx?ID=004432>.

SCSBRS126 Dueto grains are suitable for parboiled rice and based on the laboratorial analysis the soaking time during the parboiling process should be preferably 6 hours at temperatures of 60 ºC (Certificate number 0421, 3/9/2020 - LabGrãos/UFPel– Pelotas/RS), which indicates a similar parboiling behavior as other main Epagri cultivars (hydration curve can be obtained by request to corresponding author).

Added to all this, the main trait to stress out is that SCSBRS126 Dueto has the ability to tolerate cold and heat during the reproductive stage for some days, which is demonstrated by lower sterility rates compared with control cultivars (daily temperatures of 15-17 °C and night ones of 10-12 °C or daily of ≥38 °C, during 3-4 days). This was proved under controlled stress trials and also validated by two natural field cold cases (Stürmer et al. 2019, Souza et al. 2022, Marschalek et al. 2023).

SCSBRS126 Dueto is recommended to rice farmers and industry for growing in Santa Catarina pre-germinated rice areas, specially over 400 m altitude, where the yield is competitive with those of other cultivars, and the risk of lodging is low, whereas the risks of sterility due to cold are lower than the ones expected in other cultivars. On the other hand, even for the low altitude areas in Santa Catarina, SCSBRS126 Dueto remains a good option for farmers to reduce the risks of losses due to low or high temperature events at reproductive stages, like the one reported by Eberhardt (2010). It is also recommended for the Rio Grande do Sul State, at least under direct seeding system in dry soil.

MOLECULAR BACKGROUND

The genetic profile of the variety SCSBRS126 Dueto was done at the Molecular Biology Laboratory (EEI) using 26 microsatellite rice markers, using Jaccard coefficient and UPGMA agglomerative coefficient using the software NTSYS-PC 2.1, and the SCSBRS126 Dueto similarity with the other Epagri´s varieties was about 27%. The new variety has an allele (203, for the RM-316) which is not shared with the other Epagri varieties. Similarity dendrogram including SCSBRS126 Dueto, its parents, and the most important Epagri varieties still cultivated can be obtained by request to the corresponding author.

FOUNDATION SEED PRODUCTION

SCSBRS126 Dueto is registered and protected at the Ministry of Agriculture, Livestock and Food Supply (MAPA) of Brazil (respectively 48358, 7/8/2021; 20230006, 9/20/2022 - 9/20/2037). Seeds are produced by Santa Catarina Irrigated Rice Seed Producers (Acapsa) for the 2023/2024 crop season.

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Tolerance of lowland rice (*Oryza sativa*) genotypes to heat stress at anthesis

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Abstract – Global warming is occurring all over the world and climate changes are likely to affect agriculture by raising the frequency of weather events with very high and very low temperatures, which will increase the risks for food production. This study aimed to evaluate the effect of heat stress at anthesis on the agronomic performance of modern Brazilian lowland rice genotypes. The varieties SCS124 Sardo, SCS122 Miura, SCS121 CL, SCS116 Satoru, SCSBRS Tio Taka, Epagri 109 and the inbreeds SC 491 ME, SC 676, SC 792, SC 806, SC 817, SC 849 were tested. At anthesis, plants which received heat stress treatment were transferred to a growth chamber for four days at temperatures of 38°C (day) and 30°C (night). After that, they were returned to a greenhouse, remaining there until harvest, under temperatures of about 25°C. Spikelet sterility ranged from 4.0 to 84.1%. Yield varied from 10.2 to 101.2g pl^{-1}. Heat stress increased spikelet sterility and decreased grain production. The inbreeds SC 817 and SC 806 showed the lowest percentage of spikelet sterility and the highest yield under thermic stress and are promising genotypes to generate tolerant cultivars to high temperatures at anthesis.
Index terms: *Oryza sativa*; Anthesis; Heat stress; Sterility; Yield.

Tolerância de genótipos de arroz irrigado (*Oryza sativa*) ao estresse por calor na antese

Resumo – O objetivo deste trabalho foi avaliar o efeito do estresse térmico (calor) na antese sobre o desempenho agronômico de genótipos brasileiros modernos de arroz irrigado. As variedades SCS124 Sardo, SCS122 Miura, SCS121 CL, SCS116 Satoru, SCSBRS Tio Taka, Epagri 109 e as linhagens SC 491 ME, SC 676, SC 792, SC 806, SC 817, SC 849 foram avaliadas. Na antese, as plantas correspondentes ao tratamento de estresse por calor foram transferidas para uma câmara de crescimento por quatro dias sob temperaturas de 38°C (dia) e 30°C (noite). Em seguida, voltaram à casa de vegetação, onde permaneceram até a colheita sob 25°C aproximadamente. A esterilidade de espiguetas variou de 4,0 a 84,1%. A produtividade variou de 10,2 a 101,2g pl^{-1}. O estresse térmico aumentou a esterilidade das espiguetas e diminuiu a produção de grãos. As linhagens SC 817 e 806 apresentaram menor porcentagem de esterilidade de espiguetas e maior produção sob estresse de calor, sendo, portanto, genótipos promissores para gerar cultivares tolerantes a altas temperaturas na antese.

Termos para indexação: *Oryza sativa*; Antese; Estresse por calor; Esterilidade; Produtividade.

Introduction

Global warming is occurring all over the world and the Intergovernmental Panel on Climate Change (IPCC) observed a 0.3ºC rise in temperatures every 10 years. Some studies indicate that if temperatures increase by 1ºC, rice yields would decrease by 10% (BUU et al., 2021), whereas others estimate that the increasing temperature could reduce rice production by 41% at the end of the 21st century (KHAN et al., 2019). Heat stress has become increasingly important as a yield-limiting factor and the increased frequency of short-term but extremely high temperatures is particularly damaging (BUU et al., 2021). One of these high temperature events was reported in Santa Catarina at the 2009/2010 growing season. During eight consecutive days, in February 2010, maximum temperatures varied from 35ºC to 40ºC, which resulted in yield and quality losses in lowland rice in Santa Catarina (EBERHARDT, 2010).

High temperature negatively impacts all stages of rice growth but anthesis is the most susceptible stage. Temperatures above 35°C during anthesis cause irreversible damage to crops, harming the fertilization of spikelets (PRASAD et al., 2017, SOSBAI, 2018, BUU et al., 2021). High temperatures also inhibit photosynthesis and protein synthesis, increase respiration, and decrease enzymatic activity. These physiological changes hamper grain production and reduce crop yields (CUADRA et al., 2015, PRASAD et al., 2017). A review on heat stress tolerance in rice was recently written (BUU et al., 2021)

The damages related to high temperatures are similar to the injuries observed by the incidence of low temperatures. Both thermal stresses increase spikelet sterility and reduce pollen grain viability, directly affecting kernel development and decreasing the number of grains per panicle (SARSU et al., 2018, KHAN et al., 2019; BUU et al., 2021). Other effects of high temperature are lower assimilate partitioning in kernels and decreased grain filling stages, which can harm rice grain quality by reducing their amylose content and lead to white-back kernels (BUU et al., 2021).

Heat stress response in rice is a complicated process which is regulated

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by numerous proteins in different metabolic pathways. Moreover, the genetic basis of heat tolerance in rice is generally unknown. Heat stress usually induces the synthesis of heat shock proteins, which are universally observed in rice leaves. A cold shock protein was also induced in response to high temperatures in rice anthers. Some advances have been made in validating some heat tolerant genes (XIONG, 2013; KHAN et al., 2019)

Different strategies could be used to enhance rice tolerance to extreme temperatures, one of which is selecting genetically resistant genotypes by breeding (KHAN et al., 2019). However, temperature is an unpredictable and difficult factor to use in breeding programs to select for tolerant varieties. Nevertheless, some progress has been made, for example, in a similar way, in developing cold tolerant rice varieties in Hokkaido (SHINADA et al., 2013). The same efforts can be made for heat tolerance and therefore, it is important to design methodologies which can evaluate the tolerance of rice genotypes to heat stresses in the field and under controlled conditions. Conventional breeding is probably the best approach to dealing with heat tolerance, as transgenic efforts for this trait have failed to release any commercial cultivars in the last 20 years (BUU et al., 2021).

This study aims to evaluate the effect of controlled heat stresses during the anthesis of lowland rice genotypes to identify those which tolerate high temperatures. Heat stress is commonly defined as an increase in temperature above a threshold for a certain length of time which causes irreversible damage to the growth and development of plants, whereas the heat tolerance of a plant is its capacity to produce economical yield and show normal growth under high temperatures by adjusting their structural or metabolic properties (KHAN et al., 2019).

Material and methods

The experiment was conducted at the Itajaí Experiment Station (Epagri - Santa Catarina State Agricultural Research and Rural Extension Agency) during the 2018/19 growing season. The trial was set in buckets (experimental units) capable of conditioning 8kg of soil. The experimental design was entirely randomized. Treatments were arranged in a three-factorial grid (12x2x2) with three replications. The first factor corresponded to the genotypes; the second factor, to the application or not of thermal stress and the third factor, to the repetition of the experiment at different times.

In total, 12 genotypes were evaluated: six cultivars (Epagri 109, SCSBRS Tio Taka, SCS116 Satoru, SCS121 CL, SCS122 Miura, and SCS124 Sardo) and six inbreed lines (SC 491 ME, SC 676, SC 792, SC 806, SC 817, and SC 849). All genotypes belong to the *indica* subspecies, have late cycles, and were developed by Epagri or Epagri/Embrapa. The initial approach involved looking for genotypes which could face high temperature stresses. This initial group of genotypes was assembled considering their tolerance or susceptibility to another extreme abiotic stress, i.e., cold, in previous studies or observations. The inbreed lines had already been chosen by its good cold tolerance in trials conducted by the Epagri rice breeding program from about 2010 up to now (SOUSA et al., 2017; STÜRMER et al., 2019; MARSCHALEK et. al., 2019).

Sowing was performed in wooden boxes filled with a substrate composed of sand and clay. Five grams of seeds of each genotype were placed in lines in the boxes. After that, seeds were covered with substrate and watered. Sowing was done on 06/11/2018 and 08/14/2018 for the first and second experiment, respectively. After 10 days of seeding, eight seedlings of each genotype were transplanted to each bucket but only one plant/bucket was left in the V6 stage (COUNCE et al., 2000).

The six buckets for each genotype (in each experiment) were grown in a greenhouse at an average temperature of 25°C and average relative humidity of 70% from transplant to anthesis. At the beginning of anthesis, three buckets (out of six) from each genotype were subjected to a temperature of 38°C during the day and 30°C at night for four days, with a photoperiod of 12 hours of light (day)/12 hours of dark (night), and a relative air humidity of 55% in a growth chamber (made by Instalafrio – Pinhais – PR – Brazil). Each genotype had its three control buckets maintained during the whole crop cycle in the greenhouse. A similar methodology was developed in a study by Sarsu et al. (2018).

In this study, heat stress occurred between 10/01/2018 and 10/15/2018, and 11/12/2018 and 12/05/2018 for the first and second experiments, respectively. Stress began to be imposed in the growth chamber as panicles started emerging from the flag leaf, a characteristic observed between the end of stage R2 and the beginning of stage R3 (COUNCE et al., 2000). After heat stress was imposed, plants were returned to a greenhouse, in which they remained until maturity. Harvests were performed on 11/16/2018 and 01/20/2019 for the first and second experiments, respectively.

At the end of the crop cycle, tillers were harvested, and the panicles were manually threshed. Full spikelets were separated from empty ones using a forced air blower and subsequently counted (Contador – by Pfeuffer, Germany) and weighed. The following variables were determined: spikelet sterility, by counting full and empty spikelets and determining the percentage of empty spikelets; grain yield, by weighing full spikelets masses; 1,000-grain masses; and genotype tolerance to high temperatures at anthesis, by comparing the results of heat stress treatments with the respective three control buckets of each genotype which remained at the greenhouse.

Data were statistically evaluated by variance analysis using the Assistat Software Version 7.7 (SILVA & AZEVEDO, 2016). F values for treatment effects were considered at a 5% significance level ($P<0.05$). When significant, averages were compared by the Scott-Knott test at 5% significance level.

Results and discussion

The analysis of variance for spikelet sterility and grain production per plant indicated a significant triple interaction among genotype x replication x heat stress. That means that the genotypes responded differently according to heat stress application and experiment

repetition. Thus, there was a need to unfold the interaction to evaluate the influence of these factors for each genotype. Table 1 shows the results.

Analysis of variance showed significant double interactions between genotype x repetition, genotype x temperature (heat stress/control), and repetition x temperature (heat stress/control) for 1,000-grain masses. Experiment repetition also impacted this variable differently with or without heat stress, regardless of genotype.

Regarding the range of spikelet sterility - from 4.0 and 84.1% (Table 1a) - inbreeds SC 817 and SC 806 showed the lowest sterility values under heat stress. Their percentage of empty spikelets at harvest was smaller than 26%, varying from 7.3 to 11.5% for the SC 806. The good performance of the two inbreed lines under heat stress resembles the spikelet sterility from Stürmer et al. (2019) found when they were exposed to low temperatures at crop microsporogenesis. Besides, in field trials at high altitudes (500-600m), the six experiments conducted (from crop season 2013/2014 to 2018/2019), published at CBAI by Marschalek et al. (2015, 2017 and 2019) by Epagri indicate that these two lines usually show high yields, validating their adaptability for colder areas, i.e., under potential temperature stress. This may indicate the existence of a similar or overlapping tolerance mechanism to extreme temperatures in the reproductive period of the crop, regardless of whether it is high or low, as Jagadish et al. (2010), Xiong (2013), and Baliuag et al. (2015) reported. Therefore, the results from SC 806 are especially promising since its low spikelet sterility is even lower than the classical N22 line, reported to be 29%, which the literature recognizes as one of the genotypes which best tolerates high temperatures (KHAN et al., 2019). Other Epagri cultivars (SCS) (Table 1a) showed high levels of sterility under high stress temperatures, indicating the risk for rice production if these events occur.

For grain yield per plant (Table 1b), inbreeds SC 806 and SC 817 showed the highest grain yields (weight/plant-bucket) at heat stress treatments. These genotypes also had the lowest spikelet sterility (Table 1a), contributing to their

Table 1. (a) Lowland rice genotype spikelet sterility and (b) grain production per plant (bucket) subjected to heat stress for four days at anthesis in relation to control. Itajaí-SC, 2018/2019

Tabela 1. (a) Esterilidade de genótipos de arroz irrigado e (b) produção de grãos por planta (balde) (b) submetidos à estresse por calor na antese, durante 4 dias, em relação à testemunha (controle). Itajaí-SC, 2018/2019

a) SPIKELET STERILITY (%)

Genotypes	Stress 1[1/]	Control 1[1/]	Stress 2[2/]	Control 2[2/]
SCSBRS Tio Taka	84.1 aA*	19.0 dC	54.6 bA	31.2 cA
Epagri 109	82.7 aA	41.3 bA	34.5 bB	27.0 bA
SCS122 Miura	78.2 aA	12.9 cC	25.4 bC	10.2 cB
SC 676	71.1 aB	15.0 bC	20.2 bC	23.0 bA
SC 792	61.1 aB	10.3 cC	33.4 bB	18.0 cB
SC 491 ME	59.4 aB	19.1 bC	20.0 bC	15.8 bB
SCS121 CL	51.0 aC	9.6 cC	36.7 bB	17.9 cB
SC 849	46.5 aC	11.4 bC	25.9 bC	18.6 bB
SCS116 Satoru	41.0 aC	30.0 bB	46.0 aA	34.3 bA
SCS124 Sardo	36.3 aC	42.9 aA	30.7 bB	24.6 bA
SC 817	25.1 aD	18.3 aC	11.6 bD	9.4 bB
SC 806	7.3 bE	26.9 aB	11.6 bD	4.0 bB
CV% = 23.91				

b) GRAIN YIELD (g pl^{-1})

Genotypes	Stress 1[1/]	Control[1/]	Stress 2[2/]	Control[2/]
SC 806	101.2 aA*	63.0 bA	76.3 bA	90.0 aA
SC 817	64.3 aB	61.4 aA	75.8 aA	74.7 aA
SCS116 Satoru	56.4 bB	47.3 bB	49.3 bB	77.2 aA
SCS124 Sardo	47.6 aC	30.8 aB	41.7 aB	34.4 aB
SC 491 ME	45.8 bC	67.9 aA	82.2 aA	67.0 aA
SCS121 CL	45.3 bC	70.6 aA	59.2 bB	76.5 aA
SC 849	37.7 aC	62.2 aA	57.4 aB	63.4 aA
SC 792	30.6 bC	68.7 aA	54.7 aB	63.2 aA
SC 676	30.5 bC	72.8 aA	82.7 aA	63.5 aA
SCS122 Miura	20.9 bD	61.7 aA	62.7 aB	85.0 aA
Epagri 109	13.8 cD	37.6 bB	55.9 aB	64.3 aA
SCSBRS Tio Taka	10.2 cD	69.0 aA	35.8 bB	48.8 aB
CV% = 24.85				

[1/] First experiment repetition – sown on 06/11/2018. Heat treatment with 12 hours of light at 38°C (day)/12 hours of darkness (night) at 30°C in a growth chamber. Control at 25°C day/night in a greenhouse.

[2/] Second experiment repetition – sown on 08/14/2018. The same settings for light/darkness and temperature as the first experiment.

* Average followed by the same letter, lowercase in the line and uppercase in the column, fail to differ by the Scott-Knott test at the 5% significance level.

higher grain production. Thus, since spikelet sterility alone is an unreliable criterion to access heat tolerance - according to Khan et al. (2019) - SC806 especially shows its undoubted tolerance by having the best values for both traits.

Inbreeds SC817 and SC849 and the cultivar SCS124 Sardo showed no differences between the performed treatments (Table 1b). The other genotypes showed variation between at least one treatment (stress application or experiment repetition).

Cultivars SCS121 CL, SCSBRS Tio Taka, SCS116 Satoru, and SCS122 Miura showed susceptibility to high temperatures during anthesis (Table 1), with generally lower grain production under heat stress than their controls (Table 1b).

Regarding 1,000-grain mass variance analysis, the genotypes significantly differ, which is expected, as well as the two experiments (repetitions), the interaction between genotype and experiment repetition, and the interaction between genotype and temperature (Tables 2 and 3), in which one only genotype has a different 1,000-grain mass (higher mass under temperature stress). So, the genotypes failed to significantly differ for this variable between stressed plants and the control, showing that heat stress during anthesis was unable to reduce 1,000-grain masses, regardless of genotype. The thermic stress group failed to significantly differ from control, showing that temperatures are unable to affect 1,000-grain masses, whereas they differed in two previous experiments (1 and 2) which, nonetheless, involved a different set of genotypes and five different temperatures (SOUZA, 2020). Souza (2020) confirms that maximum temperatures of 38ºC at anthesis for four days failed to significatively modify 1,000-grain masses, which will occur at 40ºC and 42ºC.

The mass of 1,000 grains is established between the stages R4 (anthesis) and R8 stages (grain physiological maturity), according to the scale of Counce et al. (2000). Climatic conditions especially affect it during this period (SOSBAI, 2018). The plants which underwent heat stress remained for four days under high temperatures at the beginning of anthesis. After that, they returned to the greenhouse joining the controls plants. Therefore, both treatments had the same climatic conditions during grain filling, which possibly contributed to the absence of significant differences in 1,000-grain masses between heat stressed plants and the controls.

Table 2. Mass of 1,000 grains of lowland rice genotypes subjected to heat stress for four days at anthesis in relation to control. Itajaí-SC, 2018/19

Tabela 2. Massa de 1000 grãos de genótipos de arroz irrigado submetidos à estresse por calor durante quatro dias na antese, comparada com a testemunha. Itajaí-SC, 2018/2019

MASS OF 1,000 GRAINS (g)		
Genotypes	**Heat treatment[1/]**	**Control[2/]**
SC 676	27.0 aA*	26.6 aA
SC 491	27.0 aA	25.4 aB
SCS121 CL	26.9 aA	27.1 aA
SCS116 Satoru	26.8 aA	26.0 aB
Epagri 109	26.7 aA	25.3 aB
SC 806	26.3 aA	25.5 aB
SC 849	26.0 aA	27.3 aA
SC 817	25.9 aA	25.2 aB
SC 792	24.6 aB	25.2 aB
SCS124 Sardo	24.1 aB	22.1 bC
SCSBRS Tio Taka	23.9 aB	25.4 aB
SCS122 Miura	23.7 aB	24.9 aB
CV% = 5.62		

[1/] Heat treatment with 12 hours of light (day) at 38º/12 hours of darkness (night) at 30°C in the growth chamber.
[2/] Control at 25°C day/night in a greenhouse.
* Average followed by the same letter, lowercase on the line and uppercase on column, fail to differ by the Scott-Knott test at the 5% significance level.

Table 3. Average 1,000-grain mass of 12 irrigated rice genotypes subjected to heat stress for four days at anthesis in relation to control. Itajaí-SC, 2018/2019

Tabela 3. Massa média de 1000 grãos de 12 genótipos de arroz irrigado submetidos à estresse por calor na antese, em relação à testemunha. Itajaí-SC, 2018/2019

MASS OF 1,000 GRAINS (g)		
	Experiment 1[2/]	**Experiment 2[2/]**
Heat treatment [1/]	25.3 bA*	26.2 aA
Control [1/]	24.5 bB	26.6 aA
CV% = 5.62		

[1/] Heat treatment with 12 hours of light at 38°C (day)/12 hours of darkness at 30º (night) in the growth chamber. Control at 25°C day/night in the greenhouse.
[2/] Experiment 1 – sown on 06/11/2018. Experiment 2 – sown on 08/14/2018.
* Average followed by the same letter, lowercase in the line and uppercase in the column, fail to differ by the Scott-Knott test at the 5% significance level.

Regardless of genotype, the 1,000-grain masses of the second experiment (sown 08/14/2018) failed to significantly differ despite heat stress treatment (Table 3). On the other hand, in the first experiment (sown 06/11/2018), the control plants presented lower 1,000-grain masses (24.5g) than those subjected to heat

stress (25.3g). The greater sterility which heat stress produced in the first replication may have contributed to this result, leading to heavier seeds. As the panicles of the heat-treated plants had fewer fertile spikelets, there was greater availability of photoassimilates to fill the remaining grains.

In general, higher sterility percentages were observed after stressing the plants in the first experiment than in the second one (Table 1a). Conversely, grain production per plant showed an inverse response, having lower values in the first experiment (Table 1b). This behavior was unexpected because the two experiments were conducted using the same methodology in the same way. However, due to the limited space in the growth chamber, sowing dates of the first and the second experiments differed by two months. Therefore, differences in sowing dates might have influenced plant growth and development, interfering in the impact of heat stress on spikelet sterility and grain yield.

The main aim of this study was to evaluate the effect of heat stress at anthesis in different lowland rice genotypes, seeking to identify high temperature tolerant genotypes. This objective was achieved, because important differences among genotypes were observed in spikelet sterility and grain production per plant when under heat stress. The inbreeds SC806 and SC817 demonstrated high productive potential and stability in their spikelet sterility percentages, both with the application of stress and in their controls. Therefore, they can generate cultivars which tolerate high temperature at anthesis. This will be probably the case for the SC806 since field experiments in high altitudes and other studies on cold tolerance and normal field trials have successfully tested this line. Therefore, and because of other suitable agronomical, qualitative, and industrial traits, SC 806 will be released soon (for growing season 2023/2024 probably) as a new rice variety tolerant to high and low temperatures stresses at the reproductive stages.

Conclusions

The inbreed lines SC806 and SC817 showed the lowest spikelet sterility and highest grain yield per plant under four-day heat (38°C) stress at anthesis. A temperature of 38ºC fails to reduce the 1,000-grain mass of the tested lowland rice genotypes.

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