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**INDICADORES METABÓLICOS PARA IDENTIFICAÇÃO DE GENÓTIPOS DE
ALGODOEIRO TOLERANTES A SALINIDADE**

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Tese apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal da Paraíba, como parte dos requisitos exigidos para obtenção do título de Doutor em Agronomia.

Orientadora: Profa. Dra. Roseane Cavalcanti dos Santos

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RESUMO GERAL

A salinidade é um estresse abiótico global que afeta o desenvolvimento e produção das lavouras. Quando a concentração salina do solo ultrapassa os limites relativos a cada faixa de salinidade limiar das lavouras, os efeitos adversos, osmóticos e de íon-específico da absorção de sais, afetam diretamente o metabolismo das plantas, inibindo a absorção da água pela redução da pressão osmótica externa. Como consequência, decorrem modificações fisiológicas e bioquímicas das células vegetais. Para evitar danos, espécies tolerantes, engatilham diversos mecanismos de resposta, equilibrando o ajustamento osmótico apresentando habilidades que impedem, por meio de regulação, que excessivas quantidades de sais atinjam o protoplasma, tolerando assim os efeitos tóxicos e osmóticos decorrentes. O conhecimento de fontes de resistência às condições desfavoráveis, é importante para utilização nos programas de melhoramento genético da cultura. Nesse sentido o presente trabalho objetivou-se avaliar genótipos de algodoeiro para identificação de tolerância à salinidade com base em indicadores metabólicos, fisiológicos e bioquímicos. Onze genótipos de algodão adaptados aos ambientes Cerrado (savana) e semiárido foram cultivadas em casa de vegetação, em Campina Grande (BRS Seridó, BRS 286, FM966, CNPA 7MH, FMT 701, CNPA ITA 90, FMT 705, BRS Rubi, BRS 416, MT 152, BRS Acácia). O delineamento foi em blocos ao acaso, com três repetições, em esquema fatorial, usando 11 genótipos de algodoeiro e dois tratamentos (com e sem sal), Controle e Estresse- representado por regas com água normal ($0,3 \text{ dS m}^{-1}$) e salina (10 dS m^{-1}), durante 34 dias. A solução salina (NaCl) foi preparada seguindo a relação entre a condutividade elétrica da água (CEa) e as concentrações de sal ($10 \text{ meq L}^{-1} = 1 \text{ dS m}^{-1} \text{ CEa}$). Acompanhou-se o crescimento das plantas e avaliou-se as trocas gasosas com o auxílio do IRGA (Analisador de gás infravermelho), fluorescência da clorofila a com o auxílio de um aparelho fluorômetro modulado por pulso (OS5p - Opti Science), atividade das enzimas antioxidativas e prolina livre, realizadas a partir de extratos brutos de proteína total (25%). Como resultado identificamos que a salinidade impactou o crescimento das plantas, refletindo nos dados de trocas gasosas e prolina livre na maioria das cultivares, porém, BRS 286, FMT 705, BRS 416 e BRS Acácia e CNPA 7MH exibiram a capacidade de superar o efeito do estresse e ajustar osmoticamente, portanto minimizando os danos ao crescimento. Foi observado que as cultivares BRS Acácia e FMT 701 ativaram adequadamente a maquinaria de enzimas antioxidantes, a fim de favorecer seu processo de defesa durante o período de estresse salino. No aspecto de fluorescência, ambas reduziram a fluorescência inicial, indicando melhor eficiência fotossintética. BRS Acácia, no entanto, superou em ajuste osmótico, com base na prolina total que chegou a 40% em plantas estressadas. Com esse ajuste, as plantas sofreram menos com o impacto do estresse salino, demonstrado pelo menor desgaste celular para produção de polifenóis durante o estresse severo. Em relação à eliminação do radical peróxido, verificou-se que BRS Acácia e FMT 701 sequestraram 20% e 40%, respectivamente, indicando que este último obteve menor atividade antioxidante.

Palavras-chaves: complexo antioxidativo; ajustamento osmótico; tolerância ao estresse; *Gossypium hirsutum*.

MARCELINO, A. D. A. L **Indicadores metabólicos para identificação de genótipos de algodoeiro tolerantes a salinidade**:. 89f. Universidade Federal da Paraíba (Thesis Master in Agronomy), Areia, PB. 2023.

ABSTRACT

Salinity is a global abiotic stress that affects crop development and production. When the saline concentration of the soil exceeds the limits relative to each threshold salinity range of crops, the adverse osmotic and ion-specific effects of salt absorption directly affect plant metabolism, inhibiting water absorption by reducing pressure. external osmotic. As a consequence, physiological and biochemical changes occur in plant cells. To avoid damage, tolerant species trigger several response mechanisms, balancing the osmotic adjustment, presenting abilities that prevent, through regulation, excessive amounts of salts from reaching the protoplasm, thus tolerating the resulting toxic and osmotic effects. Knowledge of sources of resistance to unfavorable conditions is important for use in crop genetic improvement programs. In this sense, the present work aimed to evaluate commercial cotton cultivars to identify salinity tolerance based on physiological and biochemical analyses. Eleven commercial cotton cultivars adapted to the Cerrado (savannah) and semi-arid environments were grown in a greenhouse in Campina Grande (BRS Seridó, BRS 286, FM966, CNPA 7MH, FMT 701, CNPA ITA 90, FMT 705, BRS Rubi, BRS 416, MT 152, BRS Acácia). The design was in randomized blocks, with three replications, in a factorial scheme, using 11 cotton genotypes and two treatments (with and without salt), Control and Stress - represented by watering with normal water (0.3 dS m⁻¹) and saline (10 dS m⁻¹), for 34 days. The saline solution (NaCl) was prepared following the relationship between the electrical conductivity of water (ECa) and salt concentrations (10*meq L⁻¹ = 1 dS m⁻¹ ECa). Plant growth was monitored and gas exchange was evaluated with the aid of IRGA (Infrared Gas Analyzer), chlorophyll a fluorescence with the aid of a pulse modulated fluorometer device (OS5p - Opti Science), enzyme activity antioxidants and free proline, made from crude extracts of total protein (25%). As a result, we identified that salinity impacted plant growth, reflected in the data on gas exchange and free proline in most cultivars, however, BRS 286, FMT 705, BRS 416 and BRS Acácia and CNPA 7MH exhibited the ability to overcome the effect of stress and adjust osmotically, therefore minimizing damage to growth. It was observed that the BRS Acácia and FMT 701 cultivars adequately activated the antioxidant enzyme machinery, in order to favor their defense process during the period of salt stress. In terms of fluorescence, both reduced the initial fluorescence, indicating better photosynthetic efficiency. BRS Acácia, however, outperformed in osmotic adjustment, based on total proline which reached 40% in stressed plants. With this adjustment, the plants suffered less from the impact of salt stress, demonstrated by less cellular wear for polyphenol production during severe stress. Regarding the elimination of the peroxyl radical, it was found that BRS Acácia and FMT 701 sequestered 20% and 40%, respectively, indicating that the latter had lower antioxidant activity.

Keywords: antioxidative complex; osmotic adjustment; stress tolerance; *Gossypium hirsutum*.

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1 INTRODUÇÃO GERAL

A expansão da área agrícola no mundo tem incorporado, progressivamente, áreas sujeitas à secas frequentes e solos salinos. As regiões salinas, a propósito, apresentam um aumento anual em quase 10%, resultante de mudanças climáticas e gerenciamento ineficiente das áreas irrigadas, tais como exploração excessiva de recursos hídricos subterrâneos e práticas agrícolas inadequadas (HATAM et al., 2020).

A salinidade é um problema global do solo que prejudica gravemente a produtividade de diversas culturas. A área total de solos salinos e sódicos é de 831 milhões de hectares a nível mundial, dos quais 46 milhões de hectares estão em áreas irrigadas (FAO 2015).

A salinização e a sodificação são os principais processos de degradação do solo, causados pela deposição de íons Na^+ , que impactam diretamente na produção agrícola, segurança alimentar e sustentabilidade em regiões áridas e semiáridas. Estima-se que as perdas anuais na produtividade devido a esses fatores abióticos são de 31 milhões US\$ (FAO, 2022). A salinização secundária apresenta-se como uma problemática especialmente em regiões tropicais, onde prevalecem condições climáticas adversas como evapotranspiração e temperaturas elevadas de modo que a precipitação torna-se ineficiente para transportar sais da zona radicular das plantas (YADAV et al., 2011).

Quando a planta é exposta ao estresse salino, o metabolismo e as atividades fisiológicas são atingidos negativamente, causando alterações no desenvolvimento e produção, devido ao excesso de íons de sais e déficit hídrico. O crescimento é rapidamente afetado e o estresse osmótico é a primeira limitação experimentada nesse processo, devido à redução do potencial osmótico da solução do solo, que diminui a disponibilidade de água para planta interrompendo o transporte de nutrientes. A indisponibilidade de água também provoca o fechamento estomático, seguido da redução da transpiração, ou seja, afeta a absorção de dióxido de carbono, essencial a fotossíntese, promovendo danos ao aparelho fotossintético. Essas injúrias causam desordem nas trocas gasosas e prejudica a fotossíntese (DINIZ et al., 2020).

O prejuízo agrícola é decorrente dos efeitos adversos ocasionados nas lavouras, devido a presença de sais no meio. O balanço osmótico na relação solo-planta é alterado dependendo da concentração de íon Na^+ presente no solo, levando a uma cascata de respostas fisiológicas e bioquímicas no meio celular. Tal desequilíbrio pode provocar a retirada de água das células, levando à desidratação e redução dos volumes citosólico e vacuolar, de forma que a perda de água intracelular pode desencadear uma seca fisiológica (ABDELRAHEEM et al., 2019).

O acúmulo de íons é um dos principais fatores limitantes para o desenvolvimento e

crescimento das plantas. Em resposta ao estresse, os sistemas de transporte de íons e água através das membranas funcionam para controlar as mudanças de pressão do turgor nas células guarda e estimulam o fechamento estomático, além de causar redução da fotossíntese, deformação dos cloroplastos e alterações iônicas. Esses eventos levam a toxicidade, desequilíbrio nutricional e inibição da divisão celular (ASLAM et al., 2011; ZHANG et al., 2014). Em um trabalho conduzido com algodão (*Gossypium hirsutum* L.), ZHANG et al., (2017) avaliaram o efeito combinado de água e salinidade do solo e reportaram que a redução da fotossíntese esteve associada ao fechamento estomático, devido a menor absorção de água pelas raízes das plantas.

No aparato bioquímico, o efeito do estresse salino é rapidamente identificado com o aumento expressivo das espécies reativas de oxigênio (EROs), que levam a danos oxidativos, devido ao desbalanço entre a quantidade de EROs produzidas e a eliminação destas pelo sistema antioxidativo da planta. A consequência é o acúmulo das EROs na célula, provocando danos na membrana celular, organelas e ácidos nucleicos (NAVEED et al., 2020).

Para mitigar os danos fisiológicos decorrentes da salinidade, as plantas se ajustam osmoticamente aumentando a concentração de osmólitos no citosol, por absorção de solutos e/ou pela síntese de compostos orgânicos, auxiliando a manutenção do turgor da célula, além de promover o sequestro de Na^+ para o vacúolo ou apoplasto. O acúmulo de compostos orgânicos na célula favorece o potencial negativo da planta em relação ao solo, a fim de evitar a perda de água e promovendo a absorção (LIANG et al., 2018).

Nos aspectos bioquímico e molecular, cascatas de eventos são desencadeados visando acionar genes responsivos pelo sistema de defesa, os quais codificam proteínas que atuam integradas na fotossíntese, no transporte para o vacúolo, na síntese de osmólitos e na ativação de enzimas antioxidativas (GUO et al., 2015). Em um trabalho sobre estresse salino em algodão, Wang et al (2016) reportaram que a adição de 200 mmol de NaCl ao solo por 24h foi o suficiente para elevar os níveis de transcrição dos genes *GhCAT1* e *GhcAPX1*, que contribuíram para aumentar também a concentração das enzimas antioxidativas correspondentes.

O aumento da atividade enzimática é uma resposta direta ao acúmulo das EROs, no sentido de promover a rápida desintoxicação da célula. As principais enzimas atuantes nesse processo são superóxido desmutase (SOD), catalase (CAT) e ascorbato peroxidase (APX), que têm papel chave no processo de redução de danos oxidativos (GUPTA e HUANG, 2014; SHARMA et al., 2019b; HASANUZZAMAN et al., 2020). A SOD é a primeira a iniciar o processo de eliminação das EROs, dismutando o radical superóxido em peróxido de hidrogênio (H_2O_2), transformando um composto altamente nocivo em outro radical menos prejudicial à

planta. O H_2O_2 é convertido em H_2O pela ação das CAT e APX, sendo liberado pelo processo de transpiração (HASANUZZAMAN et al., 2020; FAROOQ et al., 2019; SHARMA et al., 2019b).

Além das enzimas, outras moléculas também contribuem nas vias de defesa das plantas com fins de minimizar danos celulares, como carotenoides, ácido ascórbico, tocoferóis, flavonoides, prolina, glutathione, entre outras (HASANUZZAMAN et al., 2020; SHARMA et al., 2019b; NASCIMENTO e BARRIGOSS, 2014; GUPTA e HUANG, 2014). Os açúcares, aminoácidos livres, prolina e compostos de amônio quaternário são responsáveis por garantir a turgidez celular, por serem de baixo peso molecular e não afetar o metabolismo das plantas (MILLER, et al., 2010; SHAHZAD et al., 2019). Os compostos fenólicos, por serem considerados produtos estáveis, têm função ampla nos processos celulares porque, além das respostas de defesa, podem promover diversos benefícios nas plantas, atuando como promotores de crescimento, otimizadores do rendimento de culturas e protetores contra os estresses ambientais (RAMAKRISHNA e RAVISHANKAR, 2011). De acordo com Sharma et al., 2019a, o aumento do conteúdo fenólico em plantas sob estresse salino está geralmente relacionado ao mecanismo de tolerância, porque auxilia na manutenção do equilíbrio redox e melhoria da sanidade da planta. Assim, os compostos fenólicos atuam como antioxidantes durante o estresse eliminando as EROs presentes no estresse oxidativo provocado pela salinidade.

De uma maneira geral, a resposta à tolerância ao estresse salino é genótipo dependente, sendo governada por vários genes que atuam de forma integrada de modo a elevar a expressão de seus respectivos transcritos, assegurando às plantas melhor adaptação nos ambientes onde o evento ocorre (PARDO et al., 1998; DIÉDHIOU et al., 2008; AGARWA et al., 2013). Sinais ambientais são primeiramente percebidos por moléculas sinalizadoras e envolvem fosforilação e desfosforilação de proteínas, metabolismo de fosfolipídios, detecção de Ca^{++} , etc., cuja superexpressão nas plantas demonstra maior tolerância ao estresse (AGARWA et al., 2013).

A abordagem de sinalização de moléculas para melhorar a tolerância ao sal foi demonstrada em tabaco transgênico pela modulação da expressão de um componente sinalizador de estresse de cálcio da levedura, proteína fosfatase dependente de Ca^{++} /calmodulina. As plantas de tabaco GM co-expressando as duas subunidades catalíticas e reguladoras desta proteína exibiram tolerância substancial ao NaCl (PARDO et al., 1998).

Em arroz, Diédhiou et al (2008) reportaram que a superexpressão de *SAPK4*, um tipo de serina treonina quinase conhecida por regular a expressão gênica responsiva ao estresse salino, resultou em melhorias na germinação, no crescimento e no desenvolvimento das plantas

crescidas sob estresse; o acúmulo de Na^+ e Cl^- foram menores, possibilitando melhor taxa de fotossíntese.

Em defesa contra a salinidade, a exclusão de Na^+ geralmente ocorre a partir de um gradiente eletroquímico sob ação da proteína de membrana (H^+ -ATPase), que promove o bombeamento de prótons H^+ para o exterior da célula, gerando um gradiente eletroquímico. Esse gradiente gera a força necessária ao antiporter Na^+/H^+ , onde acontece o transporte de um íon H^+ e, consequentemente, o transporte de um íon Na^+ contra o gradiente para o meio extracelular (BLUMWALD, 2000).

O acúmulo ou a exclusão de Na^+ ocorrem através da via SOS (Salt Overly Sensitive), onde um complexo de proteína quinase SOS3-SOS2 dependente de cálcio citosólico controla o nível, a expressão e a atividade dos transportadores de íons. Essa via é composta por três proteínas, SOS1, SOS2 e SOS3, que ao interagirem culminam na exclusão de Na^+ do citoplasma e, ainda, influenciam na ativação de outras proteínas transportadoras atuando para o restabelecimento da homeostase iônica (ZHU, 2002).

Os bancos de germoplasma são os melhores mananciais para explorar a variabilidade dos acessos quanto a tolerância à salinidade. Espécies halófitas possuem larga habilidade para se desenvolver em ambientes com elevadas concentrações salinas, enquanto que as glicófitas, maioria das plantas cultivadas, possuem maior limitação de crescimento, especialmente quando a concentração de salinidade supera 10 mM (ORCUTT e NILSEN, 2000). Em termos de potencial da água, as espécies halófitas possuem a capacidade de crescer em substratos cujo potencial oscila entre -1,0 e -2,5 MPa e, em condições extremas, a -5,0 MPa (WILLADINO e CÂMARA, 2004). Nas gliófitas, o manejo em solo com 200 mM de NaCl pode acarretar em morte das espécies sensíveis, como feijão (*Phaseolus vulgaris* L.), e perdas de até 60% na biomassa de algodão (WILLADINO e CÂMARA, 2010). Em milho (*Zea mays* L.) cultivado sob irrigação com níveis diferenciados de salinidade (0,78 a 6,25 dS m^{-1}), Feng et al., (2017), reportaram que na profundidade de 0,8 m, a produtividade diminuiu entre 2,08 e 3,01% para cada aumento de 1 dS m^{-1} ; na de 1,2 m, a redução foi de 3,53 a 3,93%.

Esses resultados tornam-se relevantes quando se considera que a maioria das lavouras comerciais são cultivadas em solos com tendência a salinidade e o manejo da irrigação, junto com a adoção de cultivares tolerantes são as estratégias mais adequadas para minimizar perdas expressivas na produtividade.

O algodoeiro com sistema reprodutivo considerado intermediário ou misto (VIDAL NETO e FREIRE, 2013), é referenciada como moderadamente tolerante a salinidade (MARCELINO et al., 2022). De acordo com Abul-Naas e Omran (1975), acessos de *G.*

barbadense são mais tolerante à salinidade do que os de *G. hirsutum* e *G. arboreum*, apesar da ampla variabilidade das cultivares comerciais reportada na literatura, dependendo da fase fenológica em que o estresse ocorre. Marcelino et al. (2022) submeteram diferentes acessos de algodão a 45 dias de irrigação com água salina ($6,0 \text{ dS m}^{-1}$) a partir da fase V4 (MARUR e RUANO, 2001), e identificaram quatro cultivares brasileiras tolerantes a salinidade, baseando-se nas trocas gasosas e fotossíntese do germoplasma adotado. Algumas dessas cultivares, submetidas a estresse hídrico leve (RODRIGUES et al., 2012) e severo por Pereira (2017), também foram tolerantes à seca, representando um recurso genético de amplo valor agregado por deterem tolerância a dois dos principais estresses abióticos que afetam economicamente as lavouras comerciais, além das características agronômicas aceitáveis pelos produtores (*multi traits*).

Em função disso, constata-se que a fenotipagem baseada em descritores robustos é uma ferramenta valiosa para alimentar os trabalhos de melhoramento focalizados na agregação de *multi traits* comerciais com a tolerância do germoplasma a estresses ambientais. A literatura disponibiliza vários trabalhos demonstrando o potencial das trocas gasosas, das relações hídricas, dos osmorreguladores e polifenóis na identificação de plantas tolerantes a salinidade. A seleção de genitores baseada nesses descritores favorece as estimativas de progresso no melhoramento genético, tornando mais ágil o tempo previsto para consecução de novas cultivares. O presente trabalho tem como objetivo fazer uso dessas várias ferramentas para identificar genótipos de algodão tolerantes à salinidade severa, focalizando nos vários efeitos químicos e fisiológicos durante a fase reprodutiva.

2 REFERENCIAL TEÓRICO

2.1 Origem e distribuição geográfica do algodoeiro

O algodoeiro é uma planta dicotiledônea da família *Malvaceae*, gênero *Gossypium*, com espécies de ciclo anual ou perene, dos tipos herbáceo ou arbustivo, originadas da África Central, península Arábica, Paquistão e Américas (LEE, 1984).

O gênero *Gossypium* contém cerca de 50 espécies, a maioria diplóide ($2n=2x=26$) e seis alotetraplóides ($2n=4x=52$), todas distribuídas em regiões áridas e semiáridas, além de trópicos e subtropicais. As fibras de maior valor econômico são oriundas das espécies *G. hirsutum* e *G. barbadense*, ambas alotetraplóides e nativas do Novo Mundo, e *G. herbaceum* e *G. arboreum*, ambas diploides e nativas do Velho Mundo (WENDEL e VIOT, 2003). O Brasil é centro de origem de *Gossypium mustelinum*, o que lhe confere larga variabilidade de algodoeiros tetraploides nos bancos de germoplasma nacionais (FREIRE, 2000). As demais espécies são silvestres, valioso recurso genético para futuras pesquisas prospectivas.

Alguns autores acreditam que as espécies alotetraplóides originaram-se a partir da hibridação interespecífica entre ancestrais africanos de espécies diploides pertencentes ao genoma A e americanos do genoma D (BRUBAKER; PATERSON; WENDEL, 1999; WENDEL; CRONN, 2003). A espécie *G. barbadense* é originária do Peru e do Equador, na América do Sul. O Brasil é centro de distribuição dessa espécie, detendo as formas semidomesticadas representadas pelas variedades *G. barbadense* var. *brasiliense* e *G. barbadense* var. *barbadense*, conhecidas como “rim de boi” e “quebradinho”, respectivamente (FREIRE, 2000). A espécie *G. hirsutum*, é subdividida em duas variedades: *G. hirsutum* var. *latifolium* Hutch, que corresponde ao algodoeiro do tipo herbáceo, responsável por quase 90% das cultivares comerciais no mundo, e *G. hirsutum* var. *marie-galante* (Watt) Hutch, que corresponde ao algodoeiro-mocó, do tipo perene, detentor de um manancial de genes que permite maior adaptação a ambientes com adversidades hídricas (VASCONCELOS et al., 2018; RODRIGUES et al., 2016).

2.2 Importância econômica e cultivares comerciais de algodoeiro no Brasil

O algodoeiro é uma importante *commodity* mundial, com área de 35 milhões de ha e agronegócio que mobiliza aproximadamente US\$ 12 bilhões ano⁻¹, gerando empregos direto e indiretos para mais de 350 milhões de pessoas. O Brasil é o quinto maior produtor,

representando 3% do mercado internacional e estimativa de crescimento de 4,6% ao ano (FAO, 2022); ocupa ainda a quinta posição entre os principais exportadores de fibra, além de ser um dos maiores consumidores de algodão em pluma, segundo dados da ABRAPA, (2022). Além das fibras, outros produtos derivados das sementes possuem elevado valor comercial no agronegócio cotonícola, como óleo, largamente aceito na indústria de alimentos, e torta e farelo, ambos destinados ao mercado de rações.

Na safra de 2022, foram colhidos 6,7 milhões de t de algodão em caroço, sendo 75% produzidos na região Centro-Oeste e 22% na região Nordeste, quase que totalmente no cerrado baiano (IBGE, 2022). Na Tabela 1, encontram-se os dados de produção do algodão no Brasil. Apesar do declínio da cotonicultura na década de 80, a partir de 2000 o crescimento da lavoura cresceu gradualmente até os dias atuais, tornando o Brasil um dos maiores competidores de fibras no mercado mundial. Essa pujança é resultante da interação das novas tecnologias geradas pelas Instituições de pesquisa, incluindo cultivares geneticamente modificadas, e o alto investimento na tecnologia de produção, promovido pelos agricultores, especialmente na região de cerrados (BARROS et al., 2020)

Tabela 1. Produção, área colhida e produtividade do algodão em caroço no Brasil, safra 2022.

Regiões	Área (ha)	Produção (t)	Produtividade (t ha⁻¹)
Brasil	1.622,960	6.735,606	4.150
Norte	9.295	30.418	3.273
Roraima	2.500	7.598	3.039
Tocantins	6.795	22.820	3.358
Nordeste	330.749	1.521,725	4.601
Maranhão	27.200	118.980	4.374
Piauí	8.284	31.993	3.862
Bahia	290.368	1,361,048	4.687
Sudeste	34.248	135.196	3.948
Minas Gerais	24.769	100.287	4.049
São Paulo	9.479	34.909	3.683
Centro-Oeste	1.248,668	5.048,267	4.043
Mato Grosso do Sul	24.982	113.233	4.533
Mato Grosso	1.192,260	4.806,509	4.031
Goiás	31.426	128.525	4.090

Fonte: IBGE (2022)

No Registro Nacional de Cultivares (RNC-MAPA) encontram-se as cultivares disponíveis para manejo no território nacional. Das 179 descritas, desenvolvidas por empresas públicas e privadas, 79 são algodão transgênico, cinco são fibras coloridas e o restante de cor branca desenvolvidos por métodos de melhoramento clássico (Ministério da Agricultura,

Pecuária e Abastecimento, 2017); a maioria delas é amplamente adotada para manejo na região dos cerrados do Centro Oeste e Nordeste.

Das cultivares desenvolvidas pelas Instituições de pesquisa nas últimas duas décadas, as geneticamente modificadas (GMs) e as coloridas se constituem nas tecnologias mais competitivas na cotonicultura nacional, tanto para a lavoura extensiva quanto a agroecológica. De 2005 até o corrente ano, várias cultivares de algodão GMs foram introduzidas no Brasil, algumas das quais são parentais das várias cultivares brasileiras. A maioria detém genes *Cry*, oriundos de *Bacillus thuringiensis*, para controle de lagartas, e genes codificadores de glifosato e glufosinato-amônia, para tolerância a herbicidas (BARROS et al., 2020).

Com relação ao algodão colorido, as pesquisas na Embrapa iniciaram no início da década de 80, utilizando acessos do banco de germoplasma que possuíam fibras com tonalidades variando entre verde e marrom escuro. A primeira cultivar, BRS 200 Marrom (Figura 1a), foi registrada no RNC em 2001, obtida através de cruzamentos envolvendo acessos de *G. hirsutum* subsp. *marie gallant*, com fibras longas e extralongas. Em seguida, foram registrados BRS Verde (Figura 1b), BRS Rubi (Figura 1c) e BRS Safira (Figura 1d) em 2004, BRS Topázio (Figura 1e) em 2010 e BRS Jade (Figura 1f) em 2017, todos com germoplasma de *G. hirsutum* var. *hirsutum*. Dentre estes, o BRS Topázio e o BRS Jade apresentam rendimento e características de fibra semelhantes ao algodão convencional (fibra branca) (FARIAS et al., 2017; VIDAL NETO e FREIRE, 2013).



Figura 1. Coloração das fibras das cultivares comerciais de algodão colorido (BARROS et al., 2020).

2.3 Botânica do algodão

As plantas do algodoeiro possuem hábito de crescimento indeterminado, são do tipo herbáceo ou perene, com altura que varia de 1 a 3 m, dependendo da subespécie. As flores são completas, monóicas hermafroditas, com a peculiaridade de brácteas, que são folhas modificadas que surgem na base das estruturas reprodutivas. Os botões florais contêm, frequentemente, nectários que ocorrem interna ou externamente nas suas bases (LAWS, 2013; BORÉM e FREIRE, 2014). Outra peculiaridade na planta do algodoeiro é a presença de glândulas de gossipol, uma substância tóxica para animais monogástricos, que exerce a função de proteção contra a herbivoria (BORÉM e FREIRE, 2014; HENRY; PESTI; BROWN, 2001). Trata-se de um componente polifenólico de coloração amarelada, produzido pelas glândulas encontradas nas raízes, talos, folhas e sementes das plantas (BLANCO, 2008). Com fórmula $C_{30}H_{30}O_8$, o gossipol tem peso molecular de 518,55 Dalton, é cristalino, insolúvel em água, solúvel em acetona, clorofórmio, éter, e metil-etil-cetona (butanona). A maior concentração de gossipol é encontrada na parte interna das sementes, precisamente nas glândulas, representando 20 a 39%, enquanto que outros pigmentos contam apenas com 2% (SINGLETON, 1973). Algumas variedades selvagens australianas não apresentam gossipol, contudo, essa molécula tem propriedades inseticidas, podendo atuar na defesa do algodoeiro contra parasitas e alguns microorganismos (DODOU, 2005; CAI et al., 2004).

O sistema reprodutivo do algodoeiro é considerado misto, podendo ocorrer simultaneamente autofecundação ou polinização cruzada entre gametas da mesma flor. A taxa de alogamia pode variar entre 2% e 100%, dependendo da população de insetos melíferos, velocidade do vento, ou mesmo interferência humana (BORÉM e MIRANDA, 2013; SANCHEZ JUNIOR e MALERBO-SOUZA, 2004).

O fruto, conhecido como “maçã”, tem três a cinco lóculos, cada um com cerca de onze sementes. Quando o fruto amadurece totalmente, há um processo de deiscência onde as fibras tornam-se expostas e passa a ser chamado de capulho. As sementes amadurecem nesse emaranhado de fibras, podendo conter ou não línter no tegumento (BORÉM e FREIRE, 2014). Quanto completamente maduras, as sementes contêm óleo de elevado valor comercial, com percentual de 14% a 28% de seu peso seco, dependendo do genótipo.

O principal produto do algodoeiro é a fibra, que origina-se nas células epidérmicas do óvulo da planta. A maioria das células destinadas a serem línter incham na superfície de pequenos balões, no dia da abertura da flor, embora possam demorar até quatro ou cinco dias após (Figura 1). O alongamento é rápido, correspondendo a 2 mmd^{-1} , na fase de 14 a 16 dias

após a antese (KIM, 2015). Aproximadamente 15 a 30% das células epidérmicas nos óvulos do algodoeiro tornam-se línter (LEE et al., 2007). O número de línter/semente situa-se entre 12.000 e 18.000. Na Figura 2, encontra-se imagens geradas por microscopia eletrônica relativas a diferenciação das fibras a partir de tecidos epiteliais do óvulo.

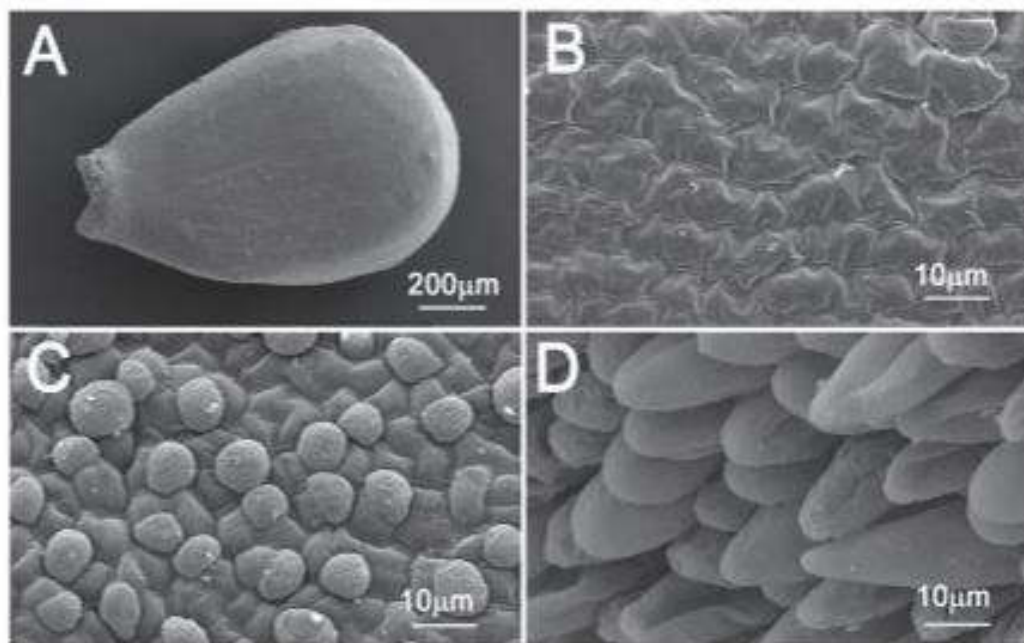


Figura 2. Imagens da diferenciação das fibras do algodão a partir de tecidos epiteliais do óvulo, obtidas por microscopia eletrônica (KIM, 2015). A- óvulo com um dias após a antese (DPA) sem iniciais de fibra detectadas. B- Imagem ampliada da superfície epidérmica do óvulo. C- Fibras iniciais se diferenciando do óvulo. D- alongamento rápido das fibras a partir de um DPA.

2.4 Ecofisiologia do algodoeiro diante da salinidade

As plantas herbáceas do algodoeiro têm ciclo que varia entre 140 e 160 dias, dependendo do genótipo. Durante seu desenvolvimento, vários eventos fisiológicos ocorrem, envolvendo simultaneamente as fases vegetativa e reprodutiva, por ser a planta de crescimento indeterminado (Figura 3). Os eventos da fase reprodutiva são os mais responsivos pela produção, de modo que as ocorrências de estresses biótico ou abiótico impactam diretamente na qualidade e rendimento das fibras.

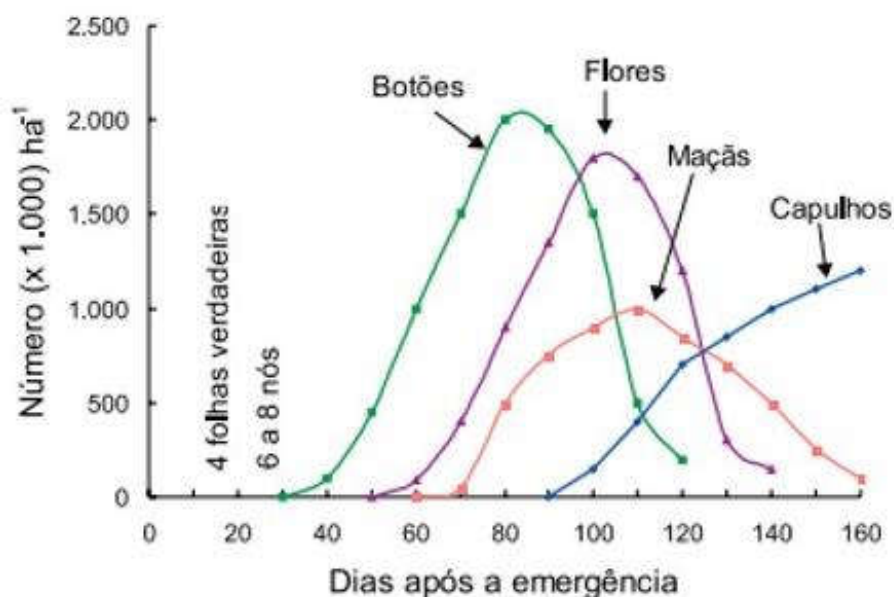


Figura 3. Eventos fisiológicos no ciclo do algodoeiro herbáceo (ROSOLEM, 2001).

Como na maioria das lavouras comerciais, o ciclo do algodoeiro é parcionado em vários estágios. Sumariamente, a fase vegetativa contém estágios representados pela letra V, que vão desde V0 - emergência até o aparecimento da primeira folha verdadeira, V1 - segunda folha verdadeira com 2,5 cm de nervura principal, até Vn, que varia em função da quantidade de ramos, comprimento das nervuras folhas e distância dos entrenós. Os estágios da fase reprodutiva são representados pelas letras B, F e C, que correspondem aos surgimentos e desenvolvimento dos botões florais, abertura das flores e dos capulhos, respectivamente (MARUR e RUANO, 2001). Na Figura 4 se apresenta um esquema representativo da ocorrência das estruturas reprodutivas, desde o surgimento do botão floral, até a maturação completa do capulho. Esses eventos ocorrem em todas espécies de *Gossypium*, com início e durações diferenciados em função das espécies e também do germoplasma avaliado.

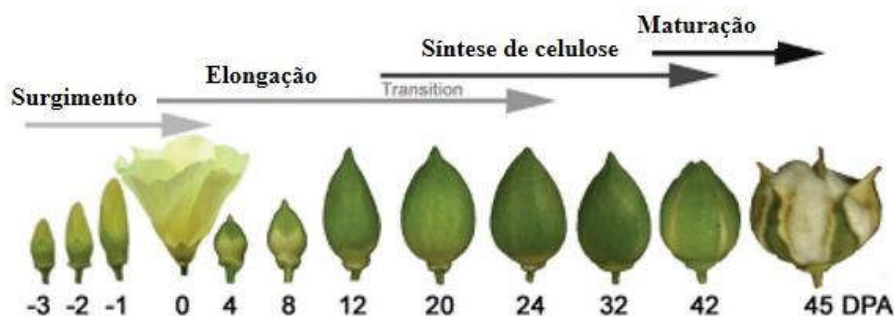


Figura 4. Esquema representativo das estruturas reprodutivas do algodoeiro (KIM, 2015).

DPA- dias após a antese.

O algodoeiro, uma planta de metabolismo fotossintético do tipo C3, se adequa a temperaturas nas faixas de 20 °C e 30 °C, precipitação anual variando entre 50 mm e 1500 mm e umidade relativa do ar em torno de 60%. Durante o manejo, a planta absorve 70% dos nutrientes após o aparecimento do primeiro botão floral e cerca de 50% de todos os nutrientes são redistribuídos na planta. A qualidade da água é imprescindível para o desenvolvimento da cultura e garantia da produtividade das fibras. As águas salobras, que contêm elevadas concentrações de sais, não permitem lixiviação adequada, impedindo absorção natural da água pelas raízes das plantas (OLIVEIRA et al., 2012; GAMA et al., 2020).

De acordo com Taiz et al. (2017), a planta do algodoeiro é capaz de crescer em ambiente com condutividade elétrica de até 5,1 dS m⁻¹. Esse limite pode variar em função do genótipo e fase fenológica da cultura (SOARES et al., 2018).

A literatura disponibiliza várias informações fisiológicas sobre tolerância de cultivares comerciais de algodão ao estresse salino, sob diferentes concentrações de sal. Lima et al. (2019) trabalhando com a cultivar colorida BRS Rubi, constataram que a irrigação com água salina nas concentrações de 5,1 a 9,1 dS m⁻¹ desencadeou uma redução na eficiência fotoquímica, partição de fotoassimilados e, conseqüentemente, na produtividade da cultura. O peso dos capulhos foi o mais prejudicado, além de causar danos à eficiência quântica do fotossistema II. Dias et al., (2020) trabalhando com a cultivar transgênica BRS 368 RF submetida a cinco níveis de salinidade (0,7 à 6,7 dS m⁻¹) durante 105 dias, observaram que a irrigação com água 0,7 dS m⁻¹ foi suficiente para comprometer o crescimento e as trocas gasosas da cultivar. A condutância estomática (*gs*) diminuiu linearmente com o aumento da salinidade apresentando perdas em torno de 60% quando comparadas com as plantas irrigadas; as taxas de transpiração (*E*) e assimilação de CO₂ (*A*) também foram afetadas pelo aumento do sal. Em outro trabalho conduzido com cultivares comerciais de algodão, Marcelino et al. (2022), submeteram as cultivares à 6,0 dS m⁻¹ durante 35 dias e conseguiram identificar três cultivares tolerantes (BRS Seridó, CNPA ITA 90 e BRS Rubi), baseando na habilidade delas de se ajustarem nos parâmetros de *gs*, *E* e *A*, no enfrentamento do estresse salino.

Com o avanço do ciclo fenológico, a resposta das cultivares de algodão podem ser diferenciadas, uma vez que alguns gatilhos fisiológicos podem ser ativados com o avanço do crescimento. No trabalho de Braz et al. (2019) que submeteram cultivares de algodão à salinidade (95 mM de NaCl) durante 72 h, iniciada na fase V3 (21 DAE), foi observado que as plantas que melhor se ajustaram ao estresse imposto reduziram a evapotranspiração e

consequentemente o consumo de água, como estratégia para diminuir a absorção de íons presentes no solo. Segundo os autores, a taxa de assimilação de CO₂ foi reduzida em todas as cultivares, com exceção de CNPA MT 152, que associado a manutenção da condutância estomática conseguiu manter bom rendimento na eficiência de carboxilação. Esse mecanismo explicou o desempenho desse material quando crescido em ambiente salino.

Outro mecanismo de grande importância é a acumulação de íons Na⁺ e Cl⁻, que ocorre quando a planta acumula íons nos vacúolos celulares, impedido que se acumulem no citoplasma, evitando danos por toxicidade. Tal resposta é indicativa de plantas tolerantes ao estresse salino. No trabalho de Sousa et al. (2010), que trabalharam com algodão sob estresse osmótico, as plantas foram irrigadas por 52 dias após a germinação com diferentes concentrações de sal (0,5, 4,0 e 8,0 dS m⁻¹), os autores observaram maior acúmulo e retenção de Na⁺ e Cl⁻ nas raízes e limbos foliares, menores alterações nos teores de K⁺ e aumento nos teores de prolina, quando comparadas às demais espécies trabalhadas (sorgo, feijão), que apresentaram um limiar de salinidade inferior ao algodão. Sob estresse severo de sal (200 mM de NaCl) e estresse hídrico, Ibrahim et al. (2019) encontraram aumento expressivo de prolina na cultivar de algodão tolerante (104,6%) e apenas 39% no sensível, comparando à media do controle, demonstrando que a adequada osmoregulação desse iminoácido é um potente indicador de tolerância a estresses ambientais para várias espécies vegetais.

Focalizando no aspecto oxidativo, Zhang et al. (2013) reportaram que, em cultivar tolerante a salinidade, a composição de ácidos graxos é mais estável e a atividade das enzimas antioxidativas é mais previsível, com maior *input* da SOD para minimizar danos celulares, facilitando o menor esforço das CAT e POX na redução de espécies oxidativas; na cultivar sensível o engatilhamento dessas enzimas se mostrou menos eficiente nas plantas sob estresse.

Além das enzimas antioxidativas, os flavonoides contribuem expressivamente na redução de danos celulares provocados por excesso de sal. A biossíntese desses compostos fenólicos é principalmente regulada em condições de estresse severo, quando as atividades das enzimas antioxidativas diminuem, levando os flavonóides a complementar a ação de outros sistemas de eliminação de EROs.

Os flavonóides fazem parte de um sistema antioxidante "secundário", ativado após um desequilíbrio grave de EROs/REDOX, devido à depleção do sistema primário de defesa antioxidante (FERDINANDO et al., 2012). Em algodão submetido a estresse salino severo Ibrahim et al. (2019), reportam que plantas de algodão tolerantes revelam maior concentração de compostos fenólicos.

Atualmente, diante do cenário mundial de mudanças climáticas, com o avanço das áreas com baixa disponibilidade hídrica e aumento no uso de águas com elevadas concentrações de sais, é necessário investir em estudos que focalizem na mitigação dos efeitos deletérios da salinidade, associados ao desenvolvimento de cultivares tolerantes que possam contribuir para minimizar os frequentes prejuízos na frustação de safras. As várias ferramentas biológicas e químicas reportadas na literatura servem de potentes referenciais para auxiliar nos procedimentos de seleção de cultivares ou linhagens avançadas, no processo do melhoramento.

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**CAPÍTULO I: GAS EXCHANGES AND OSMOTIC ADJUSTMENT IN
COTTON PLANTS SUBMITTED TO SEVERE SALINITY STRESS**

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Gas exchange and osmotic adjustment in cotton cultivars subjected to severe salt stress

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Abstract

Salinity is harmful to crops when the concentration of soluble salts overcomes the salinity threshold of the crop, causing osmotic stress and limitations in plant growth. In this scenario, adopting tolerant cultivars is the most adequate strategy to minimize agricultural losses. However, the inheritance of tolerance depends on the genotype. From this perspective, this study assessed the tolerance to severe salt stress in 11 cotton cultivars based on gas exchange parameters and the free proline content. The cultivars were grown in a greenhouse and subjected to 34 days of saline irrigation (10 dS m^{-1}), starting 45 days after seedling emergence (B1 phase). Plant growth was monitored weekly until the end of the salt stress period. The treatments consisted of a combination of two factors: eleven cultivars associated with two electrical conductivity levels of irrigation water (ECw : 0.3 and 10.0 dS m^{-1}). The experimental design was in randomized blocks in a 11×2 factorial arrangement with three replications (66 plots), with the experimental unit consisting of one plant per plot. Salinity impacted plant growth, being reflected on the gas exchange and free proline data of most cultivars. However, BRS 286, FMT 705, BRS 416, and BRS Acácia, and CNPA 7MH withstood the effects of stress and osmotically adjusted to the salt stress conditions, thus minimizing the damage to growth. Those cultivars are the most indicated for improvement programs aiming at tolerance to salt stress based on the results found in this research.

Trocas gasosas e ajuste osmótico em cultivares de algodão submetidas a estresse salino severo

Resumo

A salinidade é danosa as lavouras quando a concentração de sais solúveis ultrapassa a salinidade limiar da cultura, gerando estresse osmótico e limitações no crescimento das plantas. A adoção de cultivares tolerantes é a estratégia mais adequada para minimizar prejuízos agrícolas, porém a herança de tolerância é genótipo-dependente. Nesse trabalho avaliou-se a tolerância ao estresse salino severo em onze cultivares de algodão, baseando-se nas trocas gasosas e teor de prolina livre. As cultivares foram crescidas em casa de vegetação e submetidas a 34 dias de irrigação salina (10 dS m^{-1}), iniciada aos 45 dias de emergência das plântulas (fase B1). O crescimento das plantas foi monitorado semanalmente até o final do período de estresse salino. Os tratamentos consistiram na combinação de dois fatores: onze cultivares, associadas a dois níveis de condutividade elétrica da água de irrigação ($0,3$ e $10,0 \text{ dS m}^{-1}$). O delineamento foi em blocos ao acaso, em esquema fatorial 11×2 , com três repetições, sendo a unidade experimental constituída por uma planta por parcela. A salinidade afetou o crescimento das plantas, refletido nos dados de trocas gasosas e prolina livre na maioria das cultivares, porém BRS 286, FMT 705, BRS 416 e BRS Acácia e CNPA 7MH demonstraram habilidade para superar o efeito do estresse e se ajustar osmoticamente, minimizando os danos no crescimento. Essas cultivares são as mais indicadas para trabalhos de melhoramento visando tolerância ao estresse salino, baseando-se nos resultados encontrados nesse trabalho.

1. Introduction

Salinity is a global soil issue that severely compromises the productivity of several crops. The worldwide extent of saline and sodic soils is 831 million ha, of which 46 million ha are in irrigated areas (FAO, 2022). However, despite those numbers, saline regions increase by nearly 10% every year due to climate change and inefficient management of irrigated areas,

especially in arid and semi-arid regions where edaphoclimatic conditions such as low rainfall, high temperatures, and high evapotranspiration rates favor salt accumulation (Yadav et al., 2011; Hatam et al., 2020).

Saline soils are those with high levels of dissolved salts and high concentrations of sodium ions adsorbed in the soil matrix (Jesus et al., 2015). Na^+ accumulation due to irrigation with saline water damages the soil's colloidal aggregates, altering the soil structure and reducing permeability, thus impacting the clay-humic complex and interfering with the absorption of elements such as Ca^{++} , Mg^{++} , and K^+ (Navarro-Torre et al., 2023).

According to Macheuposhti et al. (2017), even the use of quality water transports salts to the soil, thus increasing the initial salinity. When the concentration of salts in the external environment overcomes the tolerance threshold of crops, osmotic stress begins, increasing the difficulty in water absorption since the osmotic potential of the soil solution becomes more negative, which requires greater energy expenditure by plants. Such an imbalance can remove water from the cells, leading to dehydration and reducing the cytosolic and vacuolar volumes, so that the loss of intracellular water may set off a state of physiological drought. When the accumulation of unfavorable ions such as Na^+ is prolonged, plants suffer damage from ionic stress, manifesting cellular toxicity symptoms due to the imbalance in ionic homeostasis (Abdelraheem et al., 2019).

At the physiological level, the problems caused by salt stress include osmotic imbalance, stomatal closure, reduction in photosynthesis, deformation of chloroplasts, and ionic alterations. Such events may lead to toxicity, nutritional imbalance, and inhibition of cell division (Parida and Dias, 2005; Aslam et al., 2011; Zhang et al., 2014).

Several studies state that tolerance to salinity depends on the genotype. Cultivars of the same species may show a variation in tolerance levels as a function of physiological and biochemical adjustments that are triggered when the stress signal is established (Uma and Patil,

1996; Zhang et al., 2014). Glycophytic species have greater sensitivity to salinity compared to halophytic ones, which have defense mechanisms that give them the ability for osmotic adjustment as well as compartmentalization and ionic exclusion, thus preventing the accumulation of toxic salt levels in the cytoplasm (Parida and Dias, 2005).

The cotton plant (*Gossypium hirsutum* L.) is a glycophytic species moderately tolerant to salinity, with a threshold of 5.1 dS m^{-1} in irrigation water and 7.7 dS m^{-1} in the soil saturation extract (Ayers and Westcott, 1999). Tolerance varies with the germplasm, growth phase, and stress duration. Zhang et al. (2014) tested cultivars sensitive and tolerant to salinity using increasing salt levels (80 to 240 mM NaCl) in the nutrient solution for seven days. The authors reported an effective protection mechanism in the tolerant cultivar, including mitigation of oxidative stress, lipid peroxidation, and an adequate performance in stomatal conductance, chlorophyll, and CO_2 assimilation. Superoxide Dismutase (SOD) activity also increased in leaves and roots, indicating a greater ability to reduce the oxidative damage produced by reactive oxygen species (ROS).

In another study, Wang et al. (2017) tested different biological strategies to assess the tolerance of cultivars subjected to NaCl (0.3%) for 72 h, obtaining expressive responses via molecular tools. According to those authors, the tolerant cultivar showed some effective physiological mechanisms, e.g., the ability to regulate K^+ and Na^+ transport, higher photochemical efficiency, and better antioxidant defense capacity. However, lower ROS values were found in mid-tolerant materials, which was attributed to a higher selective absorption of K^+ over Na^+ in roots across the membranes through SOS1 (plasma membrane Na^+/H^+ antiporter), AKT1 (inward-rectifier potassium channel), and HAK5 (high-affinity K^+ transporter) transcripts during salinity stress.

In Brazil, cotton is grown in regions of semi-arid climate and in the Cerrado ecosystem, with brackish water being commonly used for irrigation in order to preserve good water sources

for other purposes. Adopting tolerant cultivars to saline environments is the most viable strategy to minimize possible losses in boll production. Also, knowing the sources of resistance to unfavorable conditions is essential for genetic improvement programs aiming at the introgression of favorable genes, with biological tools contributing significantly to selection procedures. From this perspective, aiming at contributing information about tolerance to salinity, this study assessed the behavior of a group of commercial cultivars under severe salt stress based on gas exchange and free proline data.

2. Material and methods

Germplasm and Assays

Seeds of 11 cotton cultivars (Table 1) were sown in pots (1 L) containing commercial substrate (Basaplant, Base) and maintained in a greenhouse under a 12/12 h light/dark photoperiod at 21.5 °C/43 °C (min/max) and 40%/86% relative air humidity. The seedlings were watered daily (0.3 dS m⁻¹) by maintaining 100% water retention capacity in the substrate (Oliveira et al., 2012).

The treatments consisted of a combination of two factors: eleven cultivars associated with two electrical conductivity levels of irrigation water (EC_w: 0.3 and 10.0 dS m⁻¹). The experimental design was in randomized blocks, in 11 × 2 factorial arrangement with three replications (66 plots). The experimental unit consisted of one plant per plot. At the B1 stage (45 days after emergence), one seedling was randomly selected in each plot, and the salinity treatment was initiated, lasting for 34 days. An NaCl solution was added to the soil in each pot of the stressed treatments, maintaining the water electric conductivity (EC_w) at 10 dS m⁻¹.

Growth and Gas exchange

The plant height values were recorded during four weeks in each treatment, starting after 13 days of salt stress .

The gas exchange parameters were obtained through measurements performed in the third fully expanded leaf counting from the apex, collected after five and 34 days of salt stress, corresponding to phases B2 (second bud on the first reproductive branch) and F1 (occurrence of first flower), respectively. The intercellular CO₂ concentration (C_i - $\mu\text{mol CO}_2 \text{ mol}^{-1}$), transpiration (E - $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s - $\text{mol m}^{-2} \text{ s}^{-1}$), and CO₂ assimilation rate (A - $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were estimated using an Infra-Red Gas Analyzer (IRGA, ADC BioScientific Ltd, mod. LC-Pro) under natural conditions of air temperature, CO₂ concentration, and artificial radiation of $1,200 \mu\text{mol m}^{-2} \text{ s}^{-1}$. The instantaneous carboxylation efficiency (iCE - A/C_i) [$(\mu\text{mol m}^{-2} \text{ s}^{-1}) (\mu\text{mol CO}_2 \text{ mol}^{-1})^{-1}$] and instantaneous water use efficiency ($iWUE$ - A/E) [$((\mu\text{mol m}^{-2} \text{ s}^{-1}) (\text{mmol of H}_2\text{O m}^{-2} \text{ s}^{-1})^{-1})$] were determined according to Dutra et al. (2018).

Free Proline

Proline was estimated in the leaves of cotton plants in the B2 phase using the methodology described in Bates et al. (1973). Briefly, 3% (w/v) sulfosalicylic acid was used to homogenize the leaves (100 mg). The supernatant was supplemented with ninhydrin (47 mM), phosphoric acid (0.8 M), and glacial acetic acid (0.25 M) and further heated (98 °C) for 1 h. After cooling, the mixture was extracted with 0.3 vol of toluene, and the absorbance was read at 520 nm. The accumulated proline content was estimated through a standard curve and calculated based on the fresh weight. The analyses were performed in biological and experimental triplicates.

Statistical Analyses

The data were tested for normality of distribution (Shapiro and Wilk, 1965) with subsequent analysis of variance (ANOVA) by the F-test using the software Sisvar (Ferreira, 2019). The multivariate (Principal Component Analysis - PCA) analysis was performed using the software Statistica v. 7.0 (Statsoft, 2004).

3. Results and discussion

Growth of the Cultivars under Salt Stress

Plant height was influenced by salt stress throughout plant growth, with different effects observed in the cultivars as a function of the C x S interaction (Table 2). The growth reduction was phenotypically noticeable early during the stress, especially in the cultivars BRS Seridó, BRS 286, FMT 705, and MT 152 (Figure 1). Only the cv. BRS Acácia had a virtually unaltered height throughout the assay, with a small reduction of just 4% at the end of the evaluation, after 34 days of salt stress (Figure 2). Cultivars BRS Rubi, FMT 701, and FMT 705 also remained adequate, with reductions of 10%, 11%, and 13%, respectively, which are reasonable values given they were all in the reproductive phase and the high EC_w to which they were subjected. In the literature, studies involving salinity in cotton usually report the negative effects of salt on plant growth, even at electrical conductivity values lower than those used in the present study.

Silva et al. (2017) subjected the cultivar BRS Topázio to salinity at the EC_w of 6 dS m⁻¹ for 53 to 108 days and found moderate tolerance based on the variables used. The height reduction in plants ranged from 32% to 46% after 53 and 108 days of stress, respectively. Such values are close to those obtained with the cultivars of this study, although they were tested under more severe EC_w conditions (10 dS m⁻¹). According to Souza et al. (2017), who studied the cv. BRS Topázio from emergence to early reproduction, this cultivar is tolerant to salinity and can be cultivated in soils with exchangeable sodium percentages up to 38.8%. The cv. BRS Topázio, as well as the cv. BRS Rubi used in this study, are colored-fiber cotton cultivars developed by EMBRAPA and are recommended for the semi-arid regions of Brazil (Furtado et al., 2013).

With genetically modified cotton, Dias et al. (2020) subjected the cultivar BRS 368 RF to salinity at different EC_w values (0.7 to 6.7 dS m⁻¹) for 107 days, observing growth reductions

even at the minimum concentration. According to those authors, plant height was linearly inhibited by the increase in salinity, reaching 65.13% at 6.7 dS m^{-1} at the end of the assay.

The height reduction in plants grown in saline environments is one of the first phenotypically visible symptoms due to osmotic damage or ion toxicity as a function of salt accumulation in leaves, affecting cell turgor and expansion (Acosto-Motos et al., 2017; Dias et al., 2020). The tolerant genotypes also exhibited salt stress symptoms. However, they responded by using other strategies to escape the damage caused by stress, e.g., redirecting energy to maintaining biochemical and physiological activities, in addition to the compartmentalization and exclusion of Na^+ ions (Zhang et al., 2014; Sharif et al., 2019). This response could explain the behavior of the cv. BRS Acácia in the plants that received the experimental treatments, which remained virtually similar to the control plants (Figures 1 and 2). Such a result corroborates Braz et al. (2019), who subjected the cv. BRS Acácia to 95 mM NaCl for 72 h, starting in phase V3 (21 DAE). The authors found that the cultivar reduced evapotranspiration and water consumption as a strategy to decrease the uptake of ions from the soil. This mechanism explained the good performance of this material when grown in a saline environment.

Free Proline and Gas Exchange in Cotton Cultivars Subjected to Salt Stress

Proline accumulation in the plants was assessed five days after the beginning of stress, whereas the gas exchange parameters were estimated five and 34 days after the onset of stress. Statistically significant differences were found between cultivars, salinity levels, and for the effect of the C x S interaction, indicating the cultivars responded differently to the ECw imposed and used peculiar adjustments to withstand stress (Table 3). Most cultivars showed an increase in proline accumulation (Figure 3), with peaks of 249% and 147% for BRS Seridó and BRS 286, respectively, indicating that those materials invested differently in their cell reserves to adjust osmotically since the increase in proline levels in plants under stress is a response to

maintain cell integrity and support the antioxidative defense (Macêdo et al., 2019; Per et al., 2017).

The greater the input of this osmolyte in the cell, the higher the demand of energy required to prevent cell damage. The accumulation seen in this research was close to the mean values found by Guo et al. (2020), who subjected cotton plants to different soil salinity levels using NaCl, Na₂SO₄, and Na₂CO₃ + NaHCO₃. Those authors reported a significant increase in proline accumulation, amounting to 230% in the treatment with 1.39 dS m⁻¹ (NaCl) and 264% in alkaline soil (Na₂CO₃ + NaHCO₃ 0.63 dS m⁻¹). In a study with two contrasting cotton cultivars, Zhag et al. (2014) reported that salt concentrations up to 240 mM NaCl increased the level of free proline in both cultivars, but more so in the sensitive cultivar, which was more affected by the increase in salinity.

With regard to the gas exchange parameters, the cultivars showed significant statistical differences in all variables analyzed at five and 34 days of salt stress in both treatments, with a strong effect of the C x S interaction (Figures 4 and 5). Overall, the cultivars BRS 286, FMT 705, BRS 416, and BRS Acácia were the most effective in fighting salt stress based on the results of stomatal conductance (*g_s*), CO₂ assimilation rate (*A*), instantaneous carboxylation efficiency (*iCE*), and instantaneous water use efficiency (*iWUE*), which did not differ statistically from the control in both phases analyzed. These results suggest a tolerance condition since the plants maintained the capacity of adjusting to adverse conditions with little or no reduction in the CO₂ assimilation rate (Figure 4), leading to an increase in the maintenance of the *iWUE* (Figure 5).

Another interesting result was seen in the cv. CNPA 7MH, which showed reductions of 33% and 44% in the CO₂ assimilation rate (*A*) and instantaneous carboxylation efficiency (*iCE*) in the beginning of stress, respectively. However, by the end of stress, the cultivar adjusted so as to have greater control of stomatal conductance (*g_s*) and *A*, with consequent maintenance of

iCE (Figure 5 at five days). That strategy matches the genetic base of the cultivar, which responds satisfactorily when it faces water stress situations in a semi-arid environment (Farias et al., 1997).

Cultivars BRS Seridó, FM 966, CNPA ITA 90, and BRS Rubi were the most sensitive to the EC_w adopted in this study, showing worsened physiological effects by the end of stress based mainly on the CO_2 assimilation rate, with reductions of 37%, 36%, 39%, and 42%, respectively (Figure 4). In the cultivars BRS Seridó and FM 966, the drop in A occurred as a result of high transpiration rates and reduced carboxylation efficiency over the period assessed. In a study by Marcelino et al. (2022), who subjected those same cultivars to moderate salinity (6 dS m^{-1}) for 35 days, the authors reported that the cultivars BRS Seridó, CNPA ITA 90, and BRS Rubi behaved as tolerant cultivars as a function of the adjustment seen in the g_s , E , and A of plants under stress. Under the severe stress condition used in the present study, tolerance changed due to the greater accumulation of salts in the cells, affecting the A and $iWUE$. According to the literature, the reduction in A is a response to the reduction in the osmotic potential of the soil due to the increase in the content of salts in irrigation water and the accumulation of Cl^- and Na^+ ions, which hinders compartmentalization by the cell (Zhang et al., 2014; Peng et al., 2016; Dias et al., 2020).

Cultivars FMT 701 and MT 152 were the most sensitive, exhibiting drastic reductions in the CO_2 assimilation rate of 81% and 70%, respectively, which compromised the adjustment of the other physiological descriptors (Figure 4). The result of the cv. FMT 701 was also observed by Braz et al. (2019), who subjected the same cultivar to severe salt stress (approximately 95 dS m^{-1}) for 72 h, verifying that the genotype did not adapt to irrigation water with excess salts.

Clustering of Cultivars via Principal Component Analysis (PCA)

The Principal Component Analysis was performed based on the descriptors of stressed plants, whose values and eigenvalues were employed to obtain variance estimates (eigenvalues and cumulative variation) (Table 4) in order to place the cultivars in a scatter plot. Since about 89% of the total variation was explained by the first two eigenvalues, the data could be represented in a two-dimensional scatter plot (Figure 6).

Five classification groups were identified, containing the following cultivars: I- FM 966, II- CNPA 7MH and BRS Rubi, III- MT 152, IV- BRS Seridó and FMT 701, and V- BRS 286, CNPA ITA 90, FMT 705, BRS 416, and BRS Acácia.

The composition of the groups generated shows coherence with the previously discussed results, so that the genotypes that showed a markedly sensitive behavior, e.g., FM 966 and MT 152, were assigned to unitary groups, whereas BRS 286, BRS 416, FMT 705, CNPA ITA 90, and BRS Acácia occupied the same group, showing biological proximity based on the variables adopted in this research. In the physiological context, however, cultivars FMT 705, BRS 416, BRS Acácia, and CNPA 7MH were considered the most tolerant, although they use different strategies to withstand salt stress. That is justifiable because tolerance to salinity is governed by multigenic factors that lead to plants showing varied responses when facing stress situations as a function of gene interaction (Morton et al., 2018).

In a transcriptome analysis study with salinity-tolerant and -sensitive cotton plants, Guo et al. (2015) reported that distinct metabolic pathways operate in salt-tolerant and salt-sensitive upland cotton cultivars subjected to salinity, showing different levels of expression. According to those authors, genes induced in tolerant genotypes are, at the same time, repressed in sensitive genotypes, depending on the biosynthetic network that involves the trait, including transcription factors and hormone metabolism, among others. Peng et al. (2014) found 129 unigenes that were differentially expressed in salinity-tolerant cotton genotypes, including transcription factors, in plants subjected to 24 h of exposure to 200 mM NaCl. Those results are interesting

as they show the ability of plants to reprogram in face of stress, activating new genes that may be useful candidates in assisted selection processes aiming at identifying tolerant materials.

The results presented in this study show that, although the cultivars employed in this study have high agronomic value, they differ widely regarding the level of tolerance to salinity. Hence, robust germplasm databases are recommended to serve as genetic resources in improvement programs aiming at adding biological and agronomical value to the cotton agribusiness. Based on the physiological and osmoregulatory descriptors, the genotypes BRS Acácia, BRS 286, and BRS 416 were the most tolerant to the severe salt stress condition (10 dS m⁻¹).

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Table 1. Description of cotton cultivars used in this study

Cultivar	Fiber	Owner	Cycle	Recommendation
1- BRS Seridó	White	Embrapa	Mid	SA/dry season
2- BRS 286	White	Embrapa	Mid	SA and SAV/dry season
3- FM 966	White	Bayer Crop seeds	Late	SAV/water season
4- CNPA 7MH	White	Embrapa	Earliness	SA/dry season
5- FMT 701	White	FMT	Late	SAV/dry season
6- CNPA ITA 90	White	Embrapa	Late	SAV/dry season
7- FMT 705	White	FMT	Late	SAV/water season
8- BRS RUBI	Brown	Embrapa	Earliness	SA/dry season
9- BRS 416	White	Embrapa	Mid	SA and SAV/dry season
10- MT 152	White	Embrapa	Mid	SA and SAV/dry season
11- BRS Acácia	White	Embrapa	Mid	SA, irrigated/SAV, dry season

Legend: SA: semiarid, SAV: Cerrado

Table 2. Summary of the ANOVA for the plant height (cm) of cotton cultivars subjected to salt stress. Assessment performed at 13, 20, 27, and 34 days after the beginning of treatments.

SV	DF	Mean square			
		13	20	27	34
Cultivars (C)	10	98.77 **	96.88 **	102.66 **	108.01 **
Salinity (S)	1	627.45 **	967.9 **	1258.91 **	1787.76 **
Interaction C x S	10	54.60 **	59.04 **	58.34 **	71.18 **
Blocks	2	5.39 ns	17.52 ns	9.91 ns	8.23 ns
Residual	42	6.11	6.40	6.80	7.25
Cv (%)	-	6.1	5.9	6.0	6.0

SV- Source of variation, DF- degree of freedom, ns - not significant, **- significant ($p \leq 0.01$, F-test). CV- Coefficient of variation.

Table 3. Summary of the ANOVA for proline and gas exchange parameters in the cotton cultivars subjected to salinity.

SV	DF	5 days of salt stress						
		Mean square						
		Proline	C_i	E	g_s	A	iCE	$iWUE$
Cultivars (C)	10	0.030 **	2945.0 **	0.87 **	0.0030 **	18.2 **	0.0005 **	2.34 *
Salinity (S)	1	0.110 **	6842.0 **	1.32 **	0.0003 **	157.3 **	0.0041 **	10.10 **
C x S	10	0.006 **	4822.0 **	1.18 **	0.0010 **	14.3 **	0.0002 *	2.04 *
Blocks	2	0.001	394.6	0.43	0.0008	7.3	0.0002	0.85
Residual	42	0.001	202.4	0.10	0.0003	3.1	0.0000	0.85
CV (%)	-	9.4	6.1	13.0	13.2	16.9	20.9	21.3
		34 days of salt stress						
		C_i	E	g_s	A	iCE		
Cultivars (C)	10	1943.07 **	0.215 **	0.001 **	13.44 **	0.001 *		
Salinity (S)	1	6206.06 **	4.682 **	0.011 **	193.0 **	0.007 **		
C x S	10	2277.01 **	0.480 **	0.003 **	21.60 **	0.000 **		
Blocks	2	1611.40	0.281	0.001	6.92	0.001		

Residual	42	510.43	0.063	0.000	3.34	0.000
CV (%)	-	11.6	22.9	32.3	7.3	32.7

SV- Source of variation, DF- degree of freedom, *, **- significant ($p \leq 0.05$ and $p \leq 0.01$, respectively by the F-test), C- Cultivar, S - salinity, C_i - Intercellular CO_2 concentration, E - Transpiration, g_s -Stomatal conductance, A - CO_2 assimilation rate, $iWUE$ - Instantaneous water use efficiency, and iCE - Instantaneous carboxylation efficiency.

Table 4. Eigenvalues (variance), percentages of isolated and cumulative variance of the principal components obtained from the matrix formed by the physiological parameters, and growth of cotton plant genotypes subjected to salt stress.

Component	Eigenvalue	Variance (%)	Cumulative variance (%)
PC 1	5.05	72.19	72.19
PC 2	1.15	16.47	88.66
PC 3	0.59	8.46	97.12
PC 4	0.18	2.61	99.74
PC 5	0.01	0.23	99.97
PC 6	0.00	0.02	99.99
PC 7	0.00	0.00	100.0

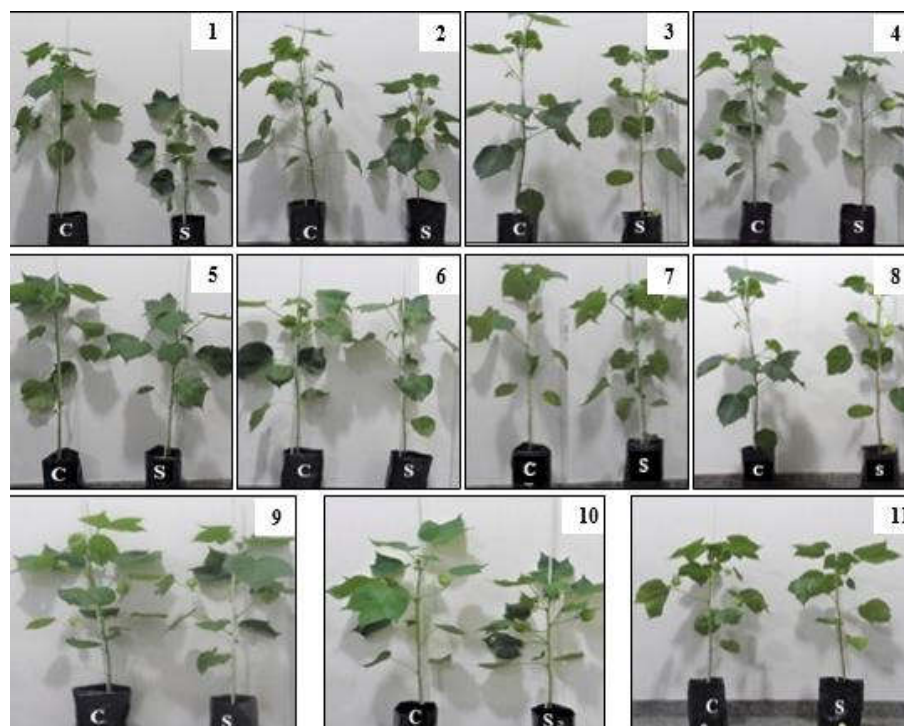


Fig 1. Profile of the cotton cultivars subjected to 13 days of severe salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7- FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11- BRS Acácia. C - control, S- stress.

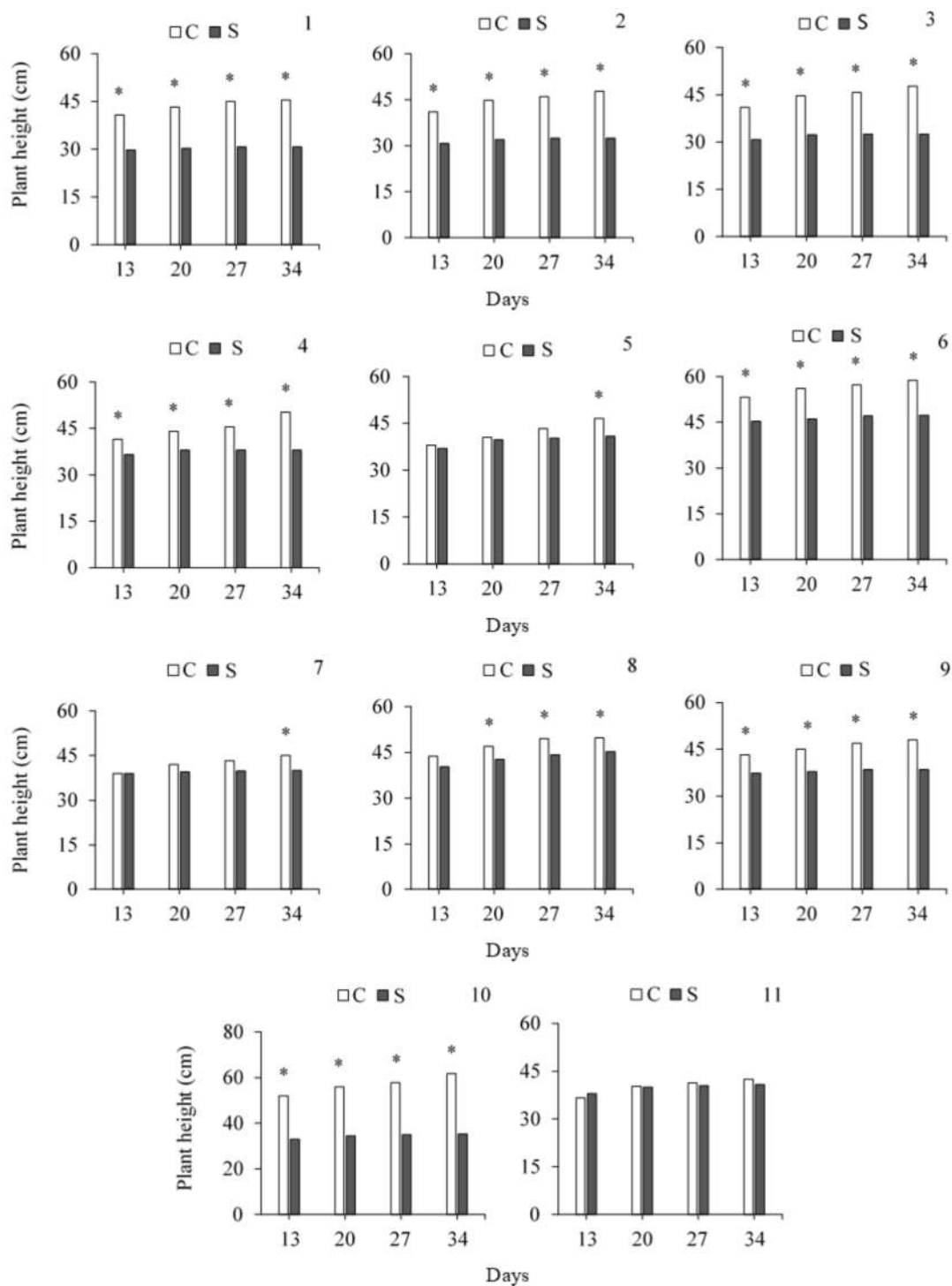


Fig 2. Height of cotton cultivars subjected to salt stress and evaluated at 13, 20, 27, and 34 days after the beginning of treatments. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7- FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11- BRS Acácia. C - control, S- stress. * between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

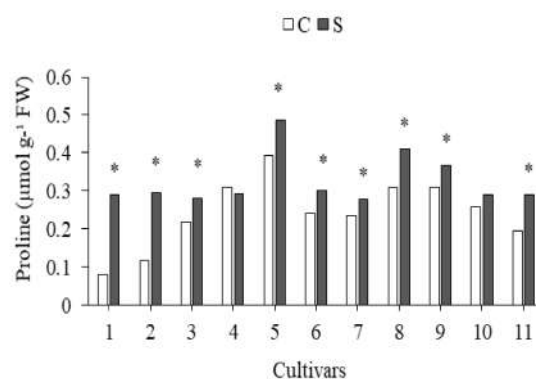


Fig 3. Accumulation of free proline in the cotton cultivars subjected to five days of severe salt stress (10 dS m^{-1}). Cultivars 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S- stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

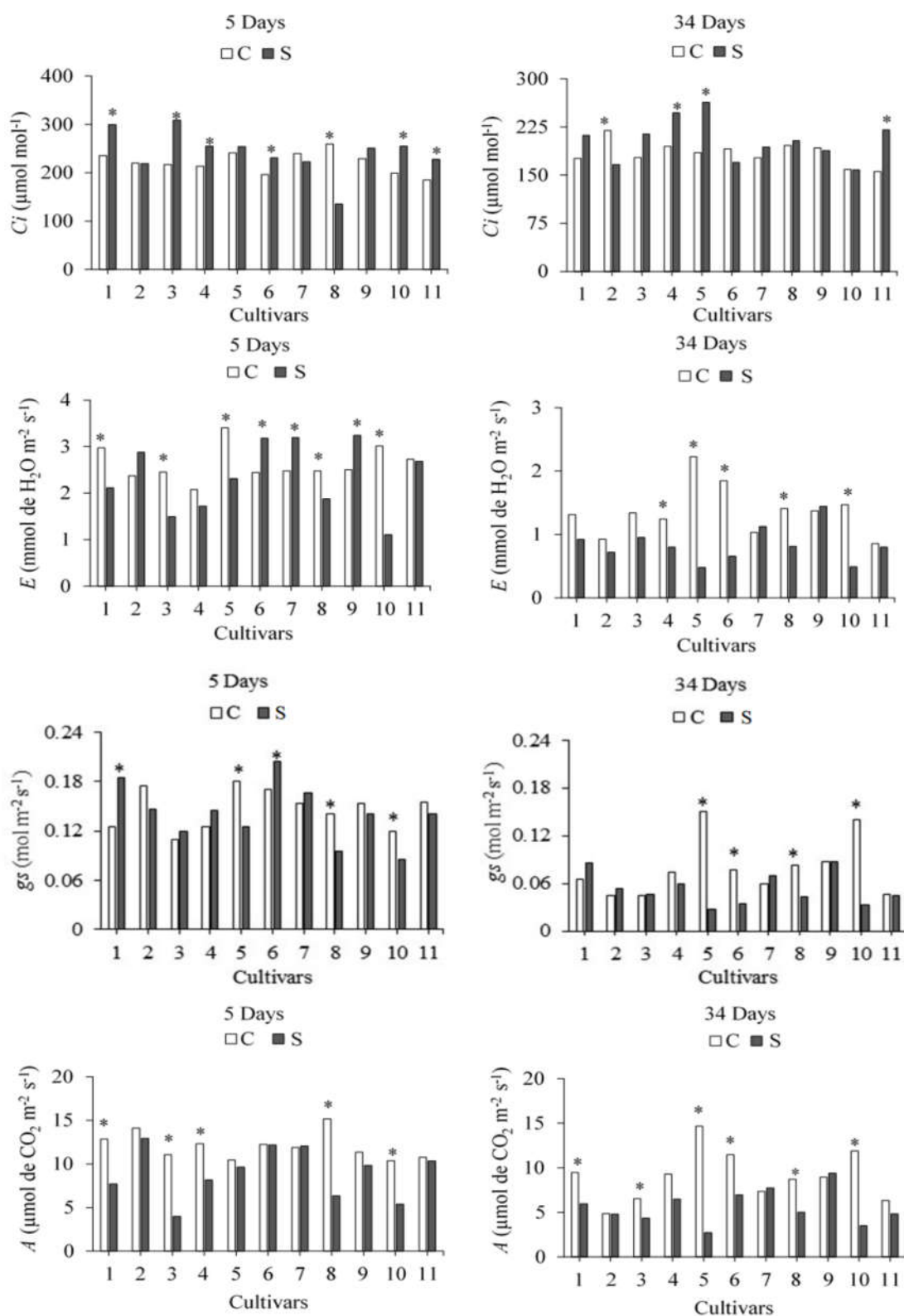


Fig 4. Gas exchange variables in the cotton cultivars subjected to five and 34 days of severe salt stress (10 dS m^{-1}). *Ci* - Internal carbon, *E* - Transpiration, *gs* - Stomatal conductance, *A* - CO_2 assimilation rate, *iCE* - Instantaneous carboxylation efficiency. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S- stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

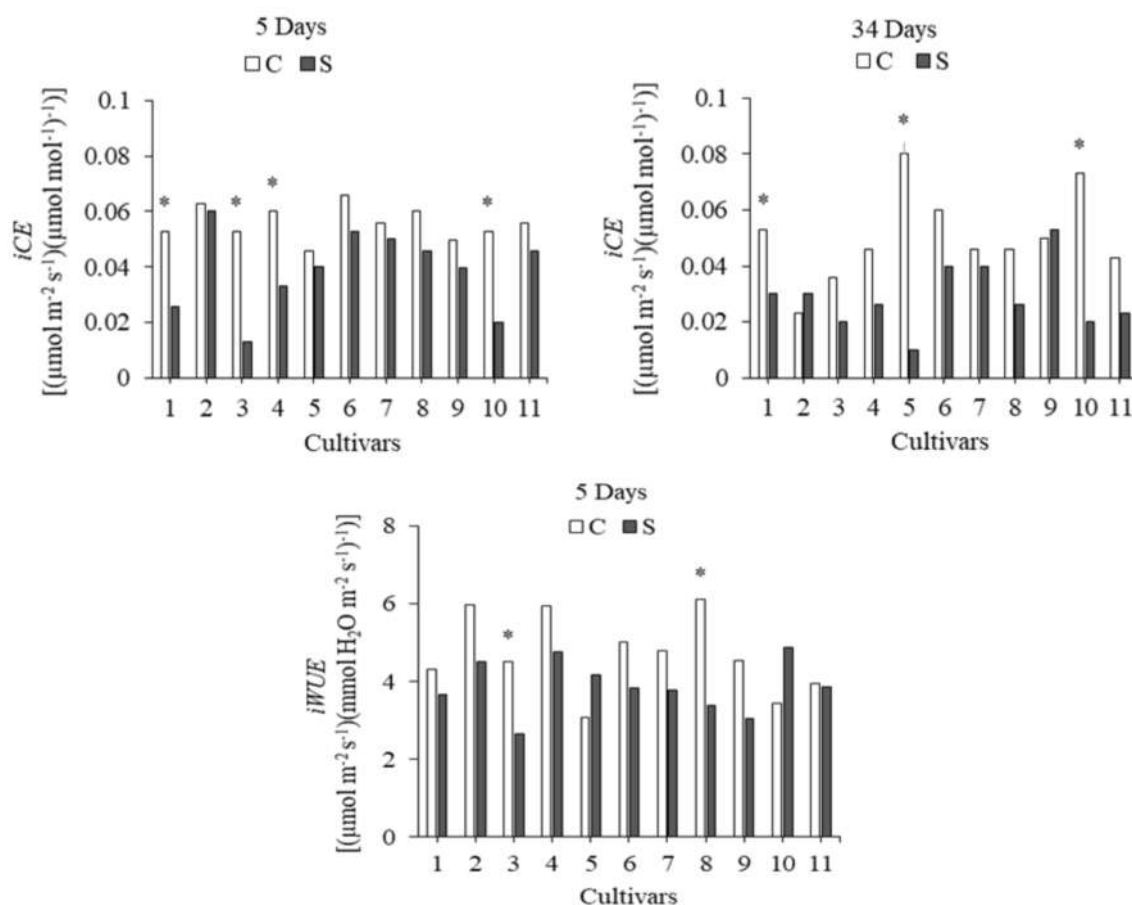


Fig 5. Instantaneous carboxylation efficiency (iCE) of cotton cultivars subjected to five and 34 days of severe salt stress and instantaneous water-use efficiency ($iWUE$) in the plants after five days of salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, 11 - BRS Acácia. C - control, S - stress. *between treatments indicates a significant statistical difference by the F-test ($p \leq 0.05$).

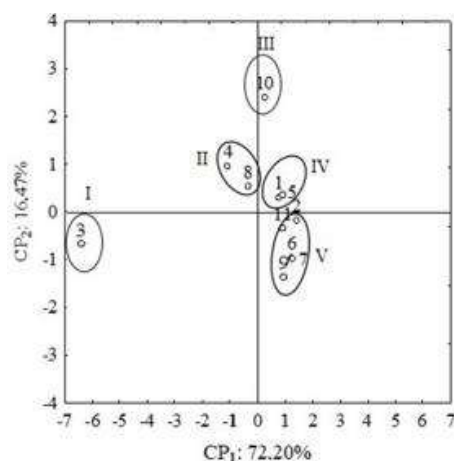


Fig 6. Scatter plot of eleven cotton cultivars subjected to salt stress. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA ITA 90, 7 - FMT 705, 8 - BRS Rubi, 9 - BRS 416, 10 - MT 152, and 11 - BRS Acácia.

**CAPÍTULO II: POLYPHENOLS AND ANTIOXIDANT PROPERTIES IN
RESPONSE TO SEVERE SALT STRESS IN COTTON**

Artigo submetido para publicação na revista Genetic Molecular Research

Polyphenols and Antioxidant Properties in Response to Severe Salt Stress in Cotton

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Abstract.

Four cotton commercial cultivars were grown in greenhouse and submitted to severe saline stress (10 dS m⁻¹), started at B1 stage, during 34 days. The random experimental design was adopted, with three repetitions. In order to identify tolerant germplasm, plants were evaluated as to chemical (antioxidative enzymes, proline, and polyphenols) and fluorescence traits. Additionally, the capacity of peroxy radical scavenging was estimated in stressed treatments, through ORAC assay. We found that BRS Acacia and FMT 701 activated adequately the machinery of antioxidant enzymes, in order to favor their defense process during salt stress period. In fluorescence aspect, both reduced the initial fluorescence, indicating better efficiency in photosynthesis processes. BRS Acácia, however, overcame in osmotic adjustment, based on total proline that reached 40% in stressed plants. With this adjustment, the plants suffered less

from the impact of saline stress, demonstrated by the lower cellular wear for polyphenol production during severe stress. Regarding the scavenging of peroxy radical, we found that BRS Acacia e FMT 701 scavenged 20% and 40%, respectively, indicating that this last showed lower antioxidant activity.

Index term: *Gossypium hirsutum*, proline, fluorescence, polyphenols, antioxidative enzymes, ROS

Abbreviations: APX- Aspartate peroxidase, CAT- Catalase, EDTA- Ethylenediaminetetraacetic acid, Nitro blue tetrazolium, ORAC- Oxygen radical absorbance capacity, SOD- Superoxide dismutase, ROS-Reactive oxidative Species

INTRODUCTION

Environmental stresses limit crop yield in any agricultural system, especially those related to water irregularities or soil salinity. To face these stresses, plants trigger different cellular responses whose ability to combat them is determined by the efficiency of the plant to sense the signals and activate its defense machinery (Dutra et al., 2018; Choudhury et al., 2019; Marcelino et al., 2022).

Water or soil salinity are the major factor limiting sustainable agriculture, spreading in irrigated land due to improper management of irrigation and drainage. Soil salinity often impacts its physical and chemical properties, with consequences for crop development. In plants, salinity causes several cell damages since salt interferes on absorption of water, leading to ionic toxicity, oxidative damages, and nutritional imbalance (Chinnusamy et al., 2006; Munns and Gilliam, 2015).

The low water availability leads to an imbalance between the formation and detoxification of reactive species, triggering biochemical stress that directly reflects on plant

phenotype (Choudhury et al., 2019). Among the reactive species, ROS (reactive oxygen species) are widely reported in plants under environmental stresses. ROS play a role as cellular signaling agents however, under conditions of environmental stress, they become highly toxic substances for the plants since the unrestricted accumulation leads to lipid peroxidation, protein oxidation, enzymatic inhibition, damage to nucleic acids, and further oxidative destruction of the cell (Dutra et al., 2018; Choudhury et al., 2019). The energetic costs associated with detoxification represent a negative aspect of ROS function at cell level. In addition to biochemical damages, the accumulation of ROS is associated with the process of degradation of chlorophylls, compromising the absorption of solar energy, which can damage the reaction centers of photosystem II, causing reduction of Fv/Fm ratio. However, the increase of antioxidant activity can alleviate oxidative stress in chloroplasts and increase the efficiency of photosystem II (Fv/Fm) (Djanaguiraman et al., 2010; Ren et al., 2021). Shaheen et al. (2012) state that traits related to photosystem II are important tools to discriminate cultivars tolerant to environmental stresses.

The main ROS are superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($HO^{\bullet}$), singlet oxygen (1O_2), peroxy radical ($ROO^{\bullet}$), hydrogen peroxide (H_2O_2), among others (Choudhury et al., 2019). The limitation of CO_2 reduction, caused by the low carboxylation capacity of the enzyme Ribulose BiPhosphatase, is reflected in oxidation and loss of electrons in the process called photorespiration. Such processes are part of the Calvin Cycle which, in C_3 plants, occurs in the cellular mesophyll. In photorespiration, superoxide or singlet oxygen and H_2O_2 are formed, which are mainly neutralized in the peroxisome by SOD and CAT enzymes in super reduced photosynthetic electron transport chain (Noctor et al., 2014).

Plants respond to ROS accumulation producing abundant enzymatic and non-enzymatic secondary metabolites that protect cells by inhibiting these destructive reactive species (You e Chan, 2015). Antioxidative enzymes are the most important components in the scavenging

system of ROS. They have key role in defense processes because are located in different subcellular compartments and work together to cell detoxification (You and Chan, 2015; Marcelino et al., 2022). SOD is the major $O_2^{\bullet-}$ scavenger and its enzymatic action results in H_2O_2 and O_2 formation. The H_2O_2 produced is then scavenged by CAT, that dismutates H_2O_2 into H_2O and O_2 , and Peroxidases (APX and GPX), that catalyze the reduction of H_2O_2 to H_2O (Rodrigues et al., 2016; You and Chan, 2015). The peroxy radicals ($ROO^{\bullet}$) are also dangerous to the cells. The $ROO^{\bullet}$ scavenging capacity is estimated by the ORAC (oxygen radical absorbance capacity) assay, which is based on the fluorescence decay by $ROO^{\bullet}$ -induced oxidation of fluorescein (Ou et al., 2001).

Among the non-enzymatic secondary metabolites, phenolic compounds (PC) are widely reported because, despite to differential response of the germplasm, the concentration of phenolic compounds generally increases when plants face environmental stresses. Some of these PCs are specific to protect the plant cells through inhibition of destructive ROS (Tapia et al., 2019; Popović et al., 2016; Hura et al., 2008). Several studies have reported that phenolic compounds, such as flavonoids and phenolic acids, act as major antioxidants, and that their accumulation in plants leads to decreased oxidative damage caused, indicating self-protection and ROS scavenging capacity in plants under abiotic stresses (Abrol et al., 2012; Ibraim et al., 2019).

Cotton (*Gossypium hirsutum* L.) is considered a moderately salt-tolerant specie, with salinity threshold level of 5.1 dS m^{-1} in electrical conductivity (EC) of the irrigation water, and 7.7 dS m^{-1} in the soil saturation extract (Ayers and Westcott, 1999). Zhang et al. (2014) reported that an increase in salinity leads to a reduction in the net photosynthetic rate (A) and stomatal conductance (g_s), as well as in dry mass and growth of cotton, with varying responses in tolerant and sensitive cultivars. For crops grown in semiarid regions, the adoption of salt tolerant

cultivars is a strategy to minimize the deleterious effects on plant growth, since the use of insufficient water for irrigation is common in the field.

Several traits have been used for identification of cotton tolerant to salinity, the most of them have provide valorous contribution to selection procedures in breeding programs. Here we spread the contribution of cellular biochemistry in the identification of tolerant plants, focusing on the concentration of total phenolic compounds and peroxy radical. Additionally, the activity of antioxidant enzymes and estimation of fluorescence were analyzed in order to better understand the responses of plant defense under salt stress.

MATERIAL AND METHODS

Genetic resources and experimental assay

Four cotton commercial cultivars (Table 1) adapted to Cerrado (savanna) and semiarid environments were grown in greenhouse, in Campina Grande, PB, Brazil (07°13' S; 53°3' W, 510 m). Seeds were sown in pots (1 L) containing commercial substrate (Basaplant, Base) and only two seedling per pot was maintained after emergence. Seedlings were watered daily (0.3 dS m⁻¹), maintaining 100% of the substrate retention capacity (Oliveira et al., 2013).

The follows treatments were applied when plants were at B1 stage (appearance of the 1st flower bud): Control and Stress- represented by watering with normal (0.3 dSm⁻¹) and saline (10 dSm⁻¹) water, during 34 days. The saline solution (NaCl) was prepared following the relationship between the water electric conductivity (EC_w) and salt concentrations (10*meq L⁻¹ = 1 dS m⁻¹ EC_w), proposed by Rhoades et al. (1992). The addition to saline water to the soil of stressed treatments maintained the EC_w at 10 dS m⁻¹. The random experimental design was adopted, with three repetitions. The wheatear conditions in greenhouse during assay were: 12:12 light/dark photoperiod, 21.5 °C / 43 °C (min / max temperature), and 40% / 86% relative humidity.

Table 1. Description of cotton cultivars used in this work.

Cultivar	Fiber	Owner	Cycle	ER
1- FM 966	White	Bayer Crop seeds	Late	SAV/water season
2- FMT 701	White	FMT	Late	SAV/dry season
3- FMT 705	White	FMT	Late	SAV/water season
4- BRS Acácia	White	Embrapa	Mid	SA, irrigated/ SAV,dry

Legend: ER- Environmental recommendation, SA: semiarid, SAV: savanna

Antioxidative activity, proline and fluorescence of cultivars under saline stress

Antioxidative enzyme assays - Total protein crude extracts (25%) were extracted from young leaves, located at mid canopy, using phosphate monobasic buffer (100 mM) and EDTA (0.1 mM), pH 7.0 (Pereira et al., 2016), and quantified through Bradford (1976) method, at 595 nm. Leaves were collected of control and stressed plants, from 5th day of starting the salt treatment. The activity of antioxidative enzymes was estimated according methodologies described in Bulbovas et al. (2005) for SOD, Azevedo et al. (1998) for CAT, and Nakano and Asada, (1981) for APX.

SOD procedure was followed with minor modifications. The reaction (2.0 ml: 100 mM of monobasic potassium phosphate (pH 7.8), 1 mM EDTA, 13 mM methionine, 75 mM of NBT, 1 nM of riboflavin and 40 µl of plant extract) was exposed to fluorescent light (75 W) for 15 min and read at 560 nm. For APX, the reaction (1.5 ml: 50 mM of monobasic phosphate buffer and 0.1 µM of EDTA (pH 6.0) contained 0.5 mM of ascorbate, 1 mM of H₂O₂ and 75 µM of plant extract. The activity was determined by oxidation of sodium ascorbate during 1 min, at 290 nm. CAT reaction (1.5 ml: 100 mM of monobasic phosphate buffer and 0.1 M of EDTA (pH 7.0), contained 20 mM of H₂O₂ and 50 µM of the plant extract. The activity was determined by H₂O₂ degradation in 1 min, at 240 nm.

Free proline - was estimated according to methodology described in Bates et al. (1973), at 520 nm. Fresh leaves (0.5 g) situated at mid canopy were extracted in sulphosalicylic acid (3%) and the homogenates were centrifuged at 10,000 g for 10 min. The supernatant (2 ml) was reacted with acid ninhydrin reagent (2 ml) and glacial acetic acid (2 ml) for 60 min at 100 °C. Then, the tubes were cooled at 4 °C. The reaction mixture was extracted with 4 ml of toluene and mixed vigorously during 20 s. Toluene was separated from aqueous phase and stored at room temperature. The absorbance was measured at 520 nm and proline accumulation was estimated from a standard curve using 0 -100 lg Lproline.

Fluorescence of 'a' chlorophyll - The initial fluorescence (F_o) and the quantum yield of photosystem II (F_v/F_m) were measured from expanded leaves located at mid canopy, at 5 and 34 days after the start of watering with saline water, using a pulse modulated fluorometer (OS5p- Opti Science). Before readings, leaves were pre-adapted to dark for 30 minutes (Warner and Burke, 1993).

Total polyphenol content - The determination of polyphenols was carried out in leaves of both treatments, collected at 5 dabs. The procedure was performed according to the spectrophotometric method of Folin–Ciocalteu described by Melo et al., (2015). Aliquots of 20 μ L of the standard solution (gallic acid) or leaf extracts and 100 μ L of the Folin– Ciocalteu solution (10%) were pipetted into each microplate well and, after 5 min, aliquots of 75 μ L of Na_2CO_3 (7.5%) solution were added to each well. The control was prepared by replacing the sample with distilled water. After 40 min of reaction, the absorbance was measured at 740 nm in a microplate reader (SpectraMax M3, Molecular Devices, LLC, Sunnyvale, CA, USA). The calibration curve was plotted using gallic acid as standard. Results were expressed in gallic acid equivalents ($\mu\text{g AG/mg lyophilized leaf}$).

Antioxidant activity by peroxy radical scavenging assay

The ORAC assay was carried out in leaves of both treatments collected at 15 dabs, in according to Melo et al. (2015). Aliquots of 30 μ L of the Trolox (standard), control, or leaves extracts, 60 μ L of 508.25 nM fluorescein, and 110 μ L of a solution of 76 mM AAPH were transferred to a 96-well black microplate. The solutions were diluted with 75 mM potassium phosphate buffer (pH 7.4), also used as blank. The reaction was performed at 37°C and the fluorescence decay was measured every minute for 2 h at 485/528 nm., the wavelengths of excitation and emission, respectively, using a spectrofluorimeter microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). Trolox was used as a standard and the results were expressed as μ mol Trolox equivalents per g of lyophilized leaf.

Statistical analyses

Means of traits were submitted to statistical analysis, adopting parametric and non-parametric models, depending on the adopted designs. The GENES software, version 2013.5.1 (Cruz, 2013) was adopted for the analyses.

RESULTS AND DISCUSSION

Antioxidant activity of cotton cultivars under salt stress

The antioxidant activity of cotton cultivars varied statistically in control and stressed treatments, demonstrating that each one reacted differently to the enzymatic event cascades in order to avoid further oxidative damages, excepting FM 966, which did not differ among the treatments. In general, BRS Acacia and FMT 701 were very able to activate the machinery of antioxidant enzymes, giving an expressive input on SOD activity to stimulate the beginning of neutralization processes of $O_2^{\bullet-}$ (Figure 1). A rise of 438% and 327% was found for these cultivars, respectively, at the 5th days after the beginning of the salt stress (dabs). This amount favored the catalysis of $O_2^{\bullet-}$ dismutation into H_2O_2 and O_2 , helping to minimize the cell

efforts of CAT (12% and 22%), and APX (22% and 33%), during the defense process of stressed plants.

Although SOD initiates the enzymatic antioxidant process, CAT is a major ROS scavenging enzyme seen as indispensable, with stress bringing about the production of CAT (Mittler, 2002). The increased CAT activities could be indicative of oxidative stress. Thus, based on enzymatic activity profile of BRS Acácia displayed on Figure 1, the low activity of CAT seen in stressed plants indicates that this cultivar revealed wide ability to overcome cellular damage induced by excess salt in leaf tissues.

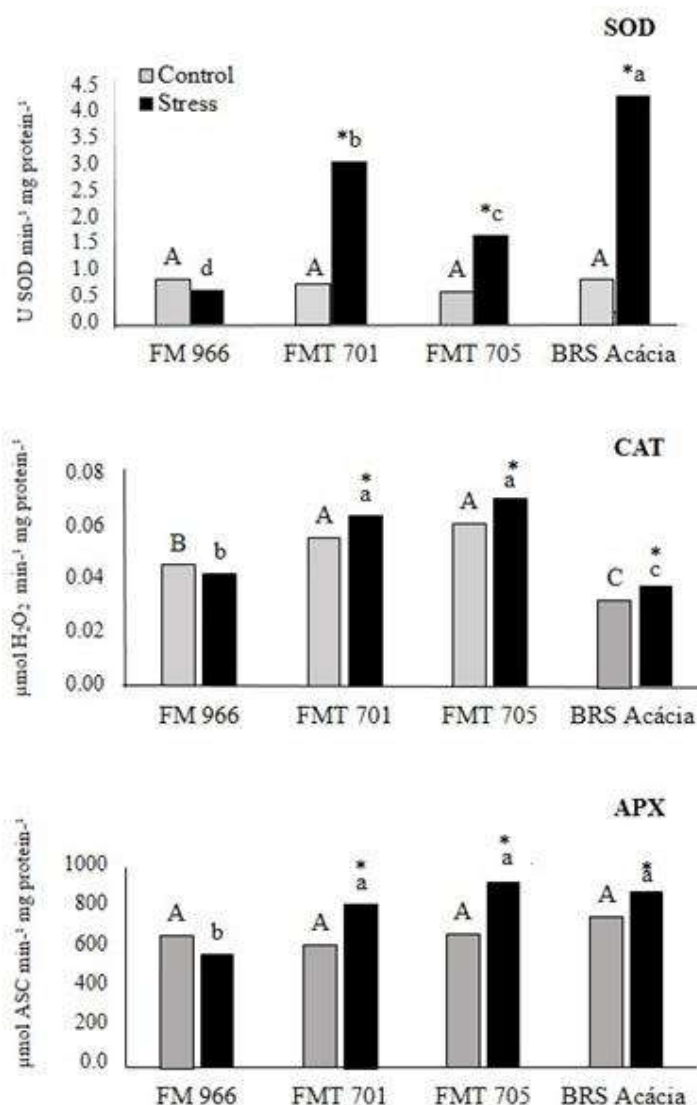


Figure 1. Antioxidative enzymes activities in leaves of cotton cultivars submitted to salt stress.

Capital and lowercase letters compare among cultivars of control and salt-stressed treatments

(Tukey, test). respectively. *; **- Means statistically different by F test at 5% and 1%, respectively (Degree of freedom of cultivar (C) x treatments (T) and Error: 3 and 16; Mean square of C x T to SOD, CAT and APX: 4.17, 0.0001, and 33060.4; Mean square of Error to SOD, CAT and APX: 0.013, 0.00001, and 5923.3).

So far, no report involving antioxidative activity of these cultivars under saline stress is available in the literature, however, with water stress, Rodrigues et al. (2016) reported that FMT 705 and FM 966 do not produce sufficient SOD input to reasonably ensure the actions of CAT and APX in the defense processes of stressed plants. This standard was also seen here (Figure 1). With FMT 701, authors reported low activity of SOD in stressed treatment and high input of APX (about 135%), at the end of antioxidative process, suggesting that the sum of peroxides not neutralized by CAT, overloaded the cell machinery, generating an expressive input of APX. The four cultivars have been endorsed in studies with water and saline stress, aiming to indicate them for genetic improvement programs, in addition to providing in the literature a better understanding of tolerance responses in materials with different levels of salt tolerance (Rodrigues et al. 2016; Marcelino et al. 2018).

Meloni et al. (2003) submitted genotypes to different salt concentrations (50, 100 and 200 mol m³ NaCl) during 21 days, and found that salinity led to a significant increase in SOD and others antioxidative enzymes in tolerant cultivars, suggesting that these genotypes may have a better protection against ROS by increasing the activity of antioxidant enzymes activities under salt stress.

Fluorescence of cultivars under saline stress

The cotton cultivars showed different behavior for fluorescence traits on different treatments, evaluated at 5 and 34 dabs. In the analysis performed at 5 dabs, we found that FMT 701 and BRS Acácia reduced the initial fluorescence (Fo) by 13.74% and 12.02%, respectively (Figure 2A), indicating that both minimized the solar energy losses, using it more efficiently

for photosynthesis processes. This inference is substantiated by the value of quantum yield of PSII (F_v/F_m), that increased by 6.9% in FMT 701, and remained statistically similar for BRS Acácia, in both control and salt stressed treatments (Figure 2C). At 34 days, these two cultivars did not differ statistically (Figure 2B and D), which is an indicative of tolerance, since negative effects on fluorescence were not found, despite the duration of salt stress. Shaheen et al. (2012) evaluated ten cotton cultivars grown under various saline concentrations, from 50 mM to 200 mM, and found that the F_v/F_m ratio in tolerant cultivar did not differ statistically between control and salt-stressed treatments, while in sensitive cultivars, the reductions of this trait were gradual due to increase in soil salinity.

It is possible to infer that the fluorescence response seen in BRS Acacia and FMT 701 was benefited by the agility of the antioxidant enzymes in minimizing further cell damages resulting from salt excess. The SOD input (Figure 1A) generated in these cultivars contributed to assisting on actions of CAT and APX to neutralize the excess reactive species in the chloroplasts, favoring an increase in the quantum yield of photosystem II. According to Djanaguiraman et al. (2010), in this situation plants are better suited to the use of solar energy, allowing greater efficiency in photosynthesis.

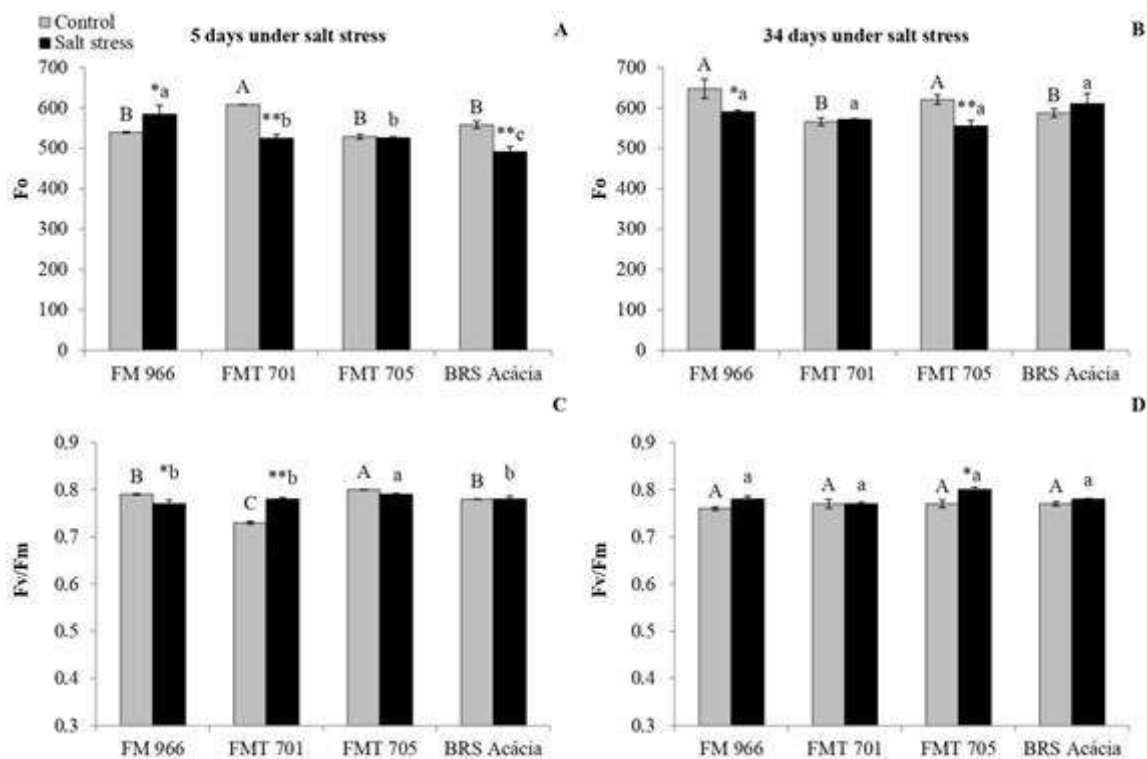


Figure 2. Initial fluorescence (Fo) and quantum yield of photosystem II (Fv/Fm) in leaves of cotton cultivars submitted to 5 (A and C) and 34 days (B and D) of salt stress. Capital and lowercase letters compare among cultivars of control and salt-stressed treatments (Tukey, test).

*, ** - Means statistically different by F test at 5% and 1%, respectively. Degree of freedom of cultivar (C) x treatments (T) and Error: 3 and 16; Mean square of C x T to Fo and Fv/Fm at 5 dabs: 5228.09 and 0.001428; Mean square of Error to Fo and Fv/Fm at 5 dabs: 339.52 and 0.000055. Mean square of C x T to Fo and Fv/Fm at 34 dabs: 2938.37 and 0.000126; Mean square of Error to Fo and Fv/Fm at 34 dabs: 724.23 and 0.000122.

Proline, total polyphenols and ORAC in cotton under salt stress

The concentration of proline, polyphenols and peroxyl radical scavenging (ORAC) in leaves of cotton cultivars were evaluated in plants of control and stressed treatments. Significant statistical differences were found for both traits in treatments, cultivars and the C x T interaction, indicating differential responses of cultivars when faced salt stress.

Proline and polyphenols were estimated at early of salt-stressed treatment, at 5 dabs, just like the antioxidative enzymes. The proline accumulation was similar to FM 966, FMT 701 and

FMT 705, reaching about 26%, while BRS Acácia accumulated 40% (Figure 3A), indicating a more reasonable osmotic adjustment in cell understanding of salt stress. Several studies have reported that proline can play several roles as an osmolyte, a ROS scavenger, and a molecular chaperone adjusting the structure of proteins, thus protecting cells from damage induced by stress (Ibrahim et al., 2019; Parida et al., 2008). Cultivars tolerant to environmental stresses often accumulates free proline in a major rate than sensitive. The pattern of BRS Acácia is an indicative of tolerance to salt stress.

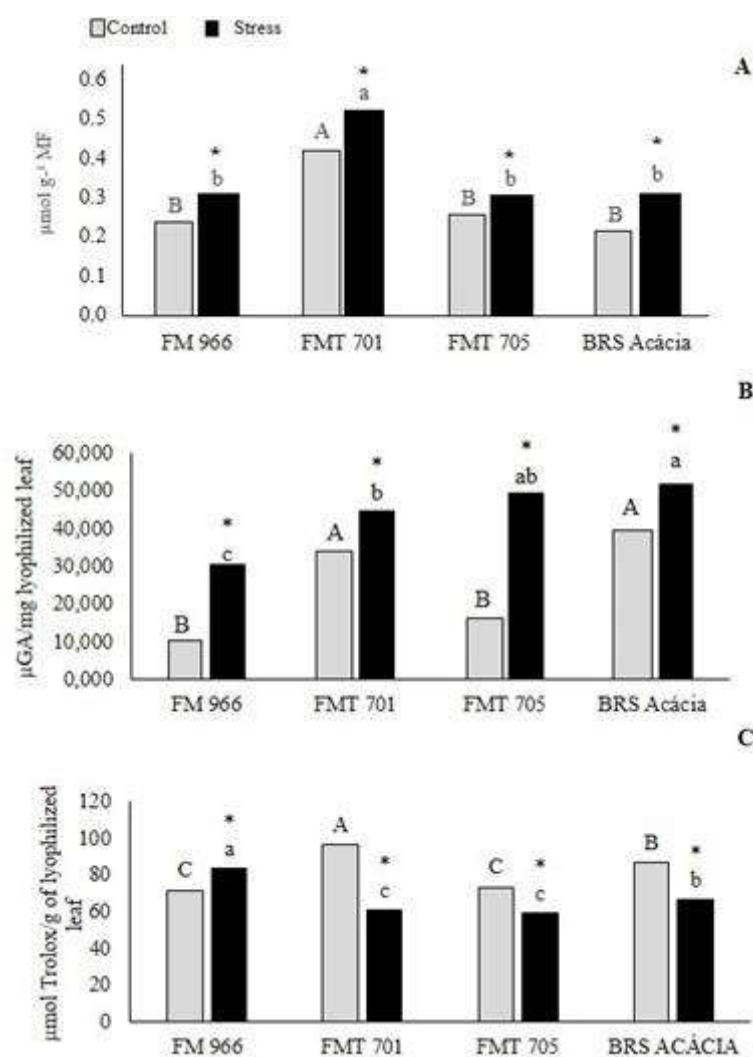


Figure 3. Proline (A), Polyphenols (B) and ORAC (C) in leaves of cotton cultivars under salt stress. Capital and lowercase letters compare among cultivars of control and salt-stressed treatments (Tukey, test). *, ** - Means statistically different by F test at 5% and 1%,

respectively. Degree of freedom of cultivar (C) x treatments (T) and Error: 3 and 16; Mean square of C x T and Error to Polyphenols (TP): 178.58 and 5.62; Mean square of C x T and Error to ORAC: 575.5 and 4.67.

To polyphenols, we found that the cell machinery of FM 966 and FMT 705 required a high load of polyphenols in order to try minimize the effects of oxidative damages, with inputs of 211% and 233% (Figure 3B); on the other hand, a minor rate was required to BRS Acácia and FMT 701, represented by only 48% and 40%. These results should not be analyzed in isolation because it depends on behavior of other organic components involved in the defense process. Comparing the rate of polyphenols found in BRS Acácia and FMT 701 with results displayed in Figure 1 (antioxidative enzymes) and Figure 3A (proline), we inferred that in face of stress signals, the defense metabolites of these cultivars acted in a coordinated manner in order to minimize further damage to cellular metabolism. Proline and polyphenols, especially, acted as main compatible solutes in order to maintain osmotic balance, to protect cellular macromolecules, to detoxify the cells, and to scavenge free radicals under stress condition.

Regarding the scavenging of peroxy radical shown in Figure 3C, we found that FM 966 was the only cultivar that showed an increase in peroxy scavenger levels (21%), in relation non salt-stressed treatment, despite having behaved as sensitive in all assays evaluated. On the other hand, for the cultivars FMT 701, FMT 705, BRS Acácia we found reductions from 40%, 20% and 20% for stressed treatment, respectively. The ORAC assay is a measure that estimates the ability of an antioxidant to quench free radicals by hydrogen donation (Prior et al., 2005.) According to the literature, there is a significant positive correlation between ORAC and total phenolic content, and it can be used in combination with other antioxidant assays to study how the general antioxidant capacity of plant tissues responds to different environmental (Dudonné et al., 2007; Gillespie et al., 2007). However, as plants use different biological tools to respond to environmental stress, it can be considered that the radical activity evaluated in this work

depended on the affinity with other components also involved in the process, interfering with the efficiency of radical scavenging. This premise is substantiated by the results of polyphenols (Figure 3B) and enzyme profile (Figure 1), especially seen in BRS Acacia, which behaved as more tolerant to severe salt stress, at concentration used in this work.

The physiological effects of salt stress in cotton are widely reported on different growth stages of cotton. Roots and boll production are more directly affected, during development, since growth relies on ionic influx in the roots along with their translocation toward shoot. According to Sharif et al., (2019), relatively lower inhibition of root growth than shoot may be attributed to lesser retention of sodium ions in the roots. In reproductive phase, delayed onset of flowering, less fruit bearing position, fruit shedding, reduced photosynthetic rate, and sucrose transformation rate are reported (Peng et al., 2016). In cotton, 60–87% synthesized sucrose is transported from subtending leaf of cotton boll to developing bolls. Under saline condition, the transportation of sucrose to these leaves towards developing bolls is retarded, resulting in reduced boll weight (Peng et al., 2016). Others organic compounds are also unbalanced during salt stress, contributing to arising of toxic effect of specific ions, such as Na⁺ and Cl⁻ ions, that interferes on absorption of other essential elements (Sá et al., 2013; Marcelino et al., 2022;).

Based on the results of this study, BRS Acacia demonstrated a behavior of tolerance to severe salinity stress, followed by FMT 701. In molecular studies using aquaporin transcripts as an identifier of salinity tolerance, Braz et al. (2019) reported that BRS Acacia, a cultivar adapted to both Brazilian semiarid and savanna environments, showed upregulation in all aquaporin isoforms, such as found in FMT 701 which is widely adapted to savanna and sensitive to salinity.

Our findings demonstrate that the biological variables adopted in this study serve as effective components to identify cotton cultivars tolerant to severe salt stress. These results are

of great contribution to the improvement work focused on salinity tolerance and open perspective to expand the understanding of these variables in other environmental stresses.

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5 CONSIDERAÇÕES GERAIS

Durante o enfrentamento do estresse salino as plantas sofrem diversos danos, mediante ao desbalanço osmótico causado pelo excesso de sal na região radicular da mesma, o desarranjo osmótico pode ocasionar seca fisiológica e desse modo a planta perder água para o meio. Além desses desarranjos fisiológicos, modificações bioquímicas afetam a organização celular, visto que mediante ao estresse, as espécies reativas de oxigênio aumentam sua concentração no espaço intracelular ocasionando danos tais como, degradação da membrana plasmática, peroxidação lipídica, danos as organelas ou até mesmo quebra da molécula de DNA e até mesmo apoptose celular. A fim de se ajustarem, mesmo estando em condições estressantes, algumas espécies acionam diversos mecanismos de defesa, sobretudo os genótipos tolerantes, dentre esses mecanismos, destacam-se, aumento de antioxidantes enzimáticos e não enzimáticos, osmorreguladores e um ajuste fisiológico eficaz. No presente trabalho baseado nos resultados obtidos, identificamos e classificamos cultivares de algodoeiro com respostas efetivas de tolerância ao estresse salino severo irrigados com água em condutividade elétrica de (10dSm^{-1}), selecionados por meio de descritores fisiológicos e bioquímicos, que se mostraram eficazes na seleção dos matérias.

A execução desse trabalho permitiu vislumbrar um leque de possibilidades de pesquisas que poderão agregar mais valor e conhecimento a respeito das cultivares comerciais BRS Seridó, BRS 286, FM 966, CNPA 7MH, FMT 701, CNPA ITA 90, FMT 705, BRS RUBI, BRS 416 e MT 152 que busquem testarem esses genótipos em campo e seguirem com avaliações em outras fases do desenvolvimento sobretudo produção, o trabalho também abre margem para testes e cruzamentos buscando agregarem novas características aos programas de melhoramento genético da Embrapa algodão.