



DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v26n4p266-273>

Multivariate classification of cotton cultivars tolerant to salt stress¹

Classificação multivariada de cultivares de algodão tolerantes ao estresse salino

Aline D. A. de L. Marcelino^{2*}, Pedro D. Fernandes³, Jean P. C. Ramos²,
Wellison F. Dutra², José J. V. Cavalcanti⁴ & Roseane C. dos Santos⁴

¹ Research developed at Campina Grande, PB, Brazil

² Universidade Federal da Paraíba, Programa de Pós-Graduação em Agronomia, Areia, PB, Brazil

³ Universidade Federal de Campina Grande, Unidade de Engenharia Agrícola, Campina Grande, PB, Brazil

⁴ Embrapa Algodão, Laboratório de Biotecnologia, Campina Grande, PB, Brazil

HIGHLIGHTS:

Salinity stress caused damage to cotton, such as reduced plant growth and changes in gas exchange, A, E, gs, EiUA, and EiC. The cultivars BRS Seridó, BRS 286, FMT 705, and BRS Rubi were classified as tolerant using physiological descriptors. FM 966 and FMT 701 showed greater sensitivity to salt stress among the cultivars tested for salt stress.

ABSTRACT: Two multivariate methods were adopted to classify salt-tolerant cotton genotypes based on their growth and physiological traits. The genotypes were cultivated in a greenhouse and subjected to 45 days of irrigation with saline water from the V4 phase onwards. Irrigation was performed with saline water with electrical conductivity (ECw) of 6.0 dS m⁻¹. A factorial-randomized block design was adopted with nine cultivars, two treatments of ECw (0.6 as the control, and 6.0 dS m⁻¹), and four replicates. Plants were evaluated for growth, gas exchange, and photosynthesis. The data were statistically analyzed using univariate and multivariate methods. For the latter, non-hierarchical (principal component, PC) and hierarchical (UPGMA) models were used for the classification of cultivars. Significant differences were found between cultivars based on univariate analyses, and the traits that differed statistically were used for multivariate analyses. Four groups were identified with the same composition in both the PC and UPGMA methods. Among them, one contained the cultivars BRS Seridó, BRS 286, FMT 705, and BRS Rubi, which were tolerant to salt stress imposed on the plants. Photosynthesis, transpiration, and stomatal conductance data were the main contributors to the classification of cultivars using the principal component method.

Key words: *Gossypium hirsutum*, osmotic stress, gas exchange, clustering

RESUMO: Dois métodos multivariados foram adotados para classificar genótipos de algodoeiro tolerantes ao sal com base no crescimento e nas características fisiológicas. Os genótipos foram cultivados em casa de vegetação e submetidos a 45 dias de irrigação com água salina, a partir da fase V4. A irrigação foi feita com água salina com CEa de 6,0 dS m⁻¹. O delineamento experimental foi em blocos casualizados com fatorial, sendo nove cultivares, dois tratamentos (controle: 0,6 e 6,0 dS m⁻¹) e quatro repetições. As plantas foram avaliadas quanto a variáveis de crescimento e fisiológicas. Os dados foram analisados estatisticamente por meio de análises univariada e multivariada. Nesse último, os modelos não hierárquicos (componentes principais, CP) e hierárquico (UPGMA) foram usados para classificação das cultivares. Diferenças significativas foram encontradas entre as cultivares com base nas análises univariadas. As variáveis que diferiram estatisticamente foram usadas para as análises multivariadas. Quatro grupos foram identificados com a mesma composição nos métodos PC e UPGMA. Entre eles, um conteve as cultivares BRS Seridó, BRS 286, FMT 705 e BRS Rubi, que se mostraram tolerantes ao estresse salino imposto as plantas. Os dados de fotossíntese, transpiração e condutância estomática foram os mais contributivos para classificação das cultivares, pelo método das componentes principais.

Palavras-chave: *Gossypium hirsutum*, estresse osmótico, trocas gasosas, agrupamento

• Ref. 253684 – Received 02 May, 2021

* Corresponding author - E-mail: alinedayanna@gmail.com

• Accepted 06 Oct, 2021 • Published 01 Nov, 2021

Editors: Geovani Soares de Lima & Hans Raj Gheyi

This is an open-access article distributed under the Creative Commons Attribution 4.0 International License.



INTRODUCTION

Salinity is a serious environmental problem affecting several crops worldwide. According to the literature, there are more than 1 billion hectares of salinized soil, mainly in arid and semiarid regions where the leaching of salt is poor due to limited and erratic rainfall (Jesus et al., 2015). Several crops are sensitive to saline soils. At the cell level, salt interferes with the absorption of water, resulting in the reduction of photosynthesis, deformation of chloroplasts, and ionic changes, leading to toxicity and nutritional imbalance (Munns & Gilliam, 2015). The intensity of osmotic stress depends on the tolerance levels of the plant species.

Cotton (*Gossypium hirsutum* L.), a glycophyte species, is considered moderately salt-tolerant, with a salinity threshold level of 5.1 dS m⁻¹ in irrigation water, and 7.7 dS m⁻¹ in the soil saturation extract (Ayers & Westcott, 1999). Abul-Naas & Omran (1975) stated that, *G. barbadense* accessions were more salinity tolerant than *G. hirsutum* and *G. arboreum* at the seedling stage, although a wide variability in salt tolerance in *G. hirsutum* has been reported in the literature.

Zhang et al. (2014) reported that an increase in salinity leads to a reduction in the net photosynthetic rate (A) and stomatal conductance (gs), 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. In general, crop breeding programs focusing on tolerance to environmental stresses are often conducted under field conditions, where hundreds of progenies are periodically evaluated for their diversity through production traits at the end of the cycle. A previous screening is performed during early vegetative growth, using biological tools to assist in the identification of responsive materials, minimizing the time and costs of routine selection procedures (Rodrigues et al., 2016; Dutra et al., 2018). As this is a laborious and extensive task, the traits must have enough weight to be responsive in the characterization procedures.

Multivariate methods are based on the simultaneous interpretation of the characteristics obtained from several genotypes. Clustering techniques are widely utilized in this process as they gather the genotypes based on a criterion that presents similarity in the behavior pattern concerning a set of traits. Thus, groups are established based on their internal homogeneity and heterogeneity (Cruz et al., 2012). Among these techniques, the hierarchical unweighted pair group method with arithmetic mean (UPGMA) is one of the most commonly used by breeders of various commercial crops (Härdle & Simar, 2003; Ramos et al., 2015).

Another method adopted to predict the similarity of genotypes is graphical dispersion by principal component analysis, which presents the clustering of genotypes through dispersion in a two- or three-dimensional plane, facilitating the identification of the most divergent types (Resende, 2007; Cruz et al., 2012).

Multivariate techniques have greatly contributed to the identification of promising genotypes when robust traits that enable discrimination of the germplasm are adopted. The groups formed act as guides that will identify the parents that should be adopted by the breeder to propagate future generations in an improved manner. In this study, the UPGMA and principal component methods were used to classify divergent cotton cultivars by salt tolerance level, by subjecting nine commercial cultivars to moderate salt stress and evaluating their growth and physiological traits.

MATERIAL AND METHODS

Nine commercial cotton cultivars (Table 1) grown in Brazil were used in this study. Assays were conducted in a greenhouse in Campina Grande, Paraíba (7°13'50" S, 35°52'52" W, 551 m) at Embrapa, the Brazilian Company of Agricultural Resource. Five seeds of each cultivar were grown in pots (35 L) containing loamy sand-Psamments soil (pH: 6.0, organic matter: 4.54 g kg⁻¹, porosity: 0.44, CEC: 2.46 cmol_c kg⁻¹), previously fertilized with NPK (12:36:52), corresponding to 16 g ammonium sulfate, 18 g mono superphosphate, and 8.6 g potassium chloride. Supplementary fertilization (100 mL) was performed as recommended by Novais et al. (1991) for assays in a greenhouse as follows: H₃BO₃ (11.11 g), CuSO₄·5H₂O (12.54 g), NaMoO₄·2H₂O (0.82 g), MnCl₂·4H₂O (31.63 g), FeCl₃·6H₂O (17 g), and ZnSO₄·7H₂O (12.54 g). After emergence, only two well-defined plants were maintained per pot.

From the V4 phase (four complete leaves; Marur & Ruano, 2001), the plants were subjected to salt stress for 35 days, maintaining an electrical conductivity of water (ECw) of 6.0 dS m⁻¹, based on a previous assay conducted by Silva et al. (2017). The ECw of the control treatment was 0.6 dS m⁻¹. The saline solution was prepared with sodium chloride (NaCl), calcium chloride (CaCl₂·2H₂O), and magnesium chloride (MgCl₂·7H₂O), at a 7:2:1 equivalent ratio of Na, Ca⁺⁺, and Mg⁺⁺, which is frequently found in irrigation waters in Northeast Brazil (Medeiros 1992). The ratio between ECw and salt concentrations (10 mmol_c L⁻¹ = 1 dS m⁻¹ ECw) established by Rhoades et al. (1992) as valid for ECw values from 0.1 to 5.0 dS m⁻¹ was used to prepare solutions, which were adjusted using a conductivity meter.

A 2-day irrigation interval was adopted to maintain soil moisture near the field capacity. The volume of water per pot was estimated based on the water requirement of cotton (Kc) using Eq. 1, established by Salassier et al. (2006), and a leaching fraction of 10%. Evapotranspiration was estimated daily using

Table 1. Details of cotton cultivars used in this study

Cultivar	BT	Source	Recommendation
1. BRS Seridó	H	Embrapa	Semiarid/dry season
2. BRS 286	H	Embrapa	Semiarid and savanna/dry season
3. FM 966	H	Bayer Crop seeds	Savanna/wet season
4. CNPA 7MH ¹	H	Embrapa	Semiarid/dry season
5. FMT 701	H	FMT	Savanna/dry season
6. CNPA 5M	A	Embrapa	Semiarid/dry season
7. CNPA ITA 90	H	Embrapa	Savanna/dry season
8. FMT 705	H	FMT	Savanna/wet season
9. BRS RUBI	H	Embrapa	Semiarid/dry season

¹ Obtained from crossing of the Marie galant and latifolium landraces. BT, Botanical type; H, Herbaceous; A, Arbustive. FMT: Mato Grosso Foundation

an evaporimeter tank. A factorial-randomized block design was adopted with nine cultivars, two electrical conductivities of irrigation water (0.6 and 6 dS m⁻¹), and four replicates (Eq. 1):

$$ETc = Et \times Kc \rightarrow LB = ETc \times A \rightarrow LL = LB - LI \quad (1)$$

where:

- ETc - crop evapotranspiration;
- Et - tank evaporation;
- Kc - crop coefficient;
- LB - total depth of water;
- A - area of the pot (m²);
- LL - net depth of water; and,
- LI - leached depth.

The maximum and minimum average temperature and relative air humidity during the assay were: 28.2–16.1 °C and 96.33–70.33%, respectively, collected from the Embrapa Weather station (<https://tempo.inmet.gov.br/TabelaEstacoes/A313>).

Growth analyses were performed every 10 days, starting on the 15th day of stress treatment. The following traits were evaluated: plant height, main stem diameter, main stem node number, and total leaf number.

Gas exchange was analyzed 25 days after salt stress in the pre-flowering stage. Stomatal conductance (gs), internal CO₂ concentration (Ci), transpiration (E), and liquid photosynthesis (A) were measured in fully expanded young leaves of three plants per cultivar per treatment, using an infrared gas analyzer (IRGA) with 1600 μmol m⁻² s⁻² light intensity, 25 ± 2 °C leaf temperature, and of 200 mL min⁻¹ air flow, between 9:30 and 11:00 a.m. The water use efficiency (EiUA) and carboxylation efficiency (EiC) were estimated through the photosynthesis and transpiration ratio, and the photosynthesis and internal carbon concentration ratio, respectively. The fluorescence data, initial fluorescence (F₀), maximum fluorescence (F_m), variable fluorescence (F_v), and potential quantum yield (F_v/F_m), were collected using a non-modular fluorescence analyzer (NMFA; PEA II, Hansatech Instruments, UK), using leaf clips placed on the young leaves located at the canopy and kept in the dark for 30 min.

Growth and physiological data were subjected to the Lilliefors-normality test and variance analysis (F test, p ≤ 0.01). Means were compared using the Scott - Knott test (p < 0.05). Two multivariate methods were adopted for the classification of cultivars: UPGMA and PCA. The coefficient of cophenetic correlation was estimated to adjust the UPGMA hierarchical method, based on Sokal & Rohlf (1962). The Euclidian distance was used to estimate the cultivar dissimilarities. The statistical procedures were performed using GENES software, version 2017.3.31 (Cruz, 2013).

RESULTS AND DISCUSSION

Statistically significant differences were found among cultivars for all traits; however, the differences were found only for plant height (PH) between treatments (ECw) (Table 2). The effects of interaction (C × ECw) were found for plant height

Table 2. Summary of analyses of variance of growth traits in cotton subjected to 35 days of salt stress

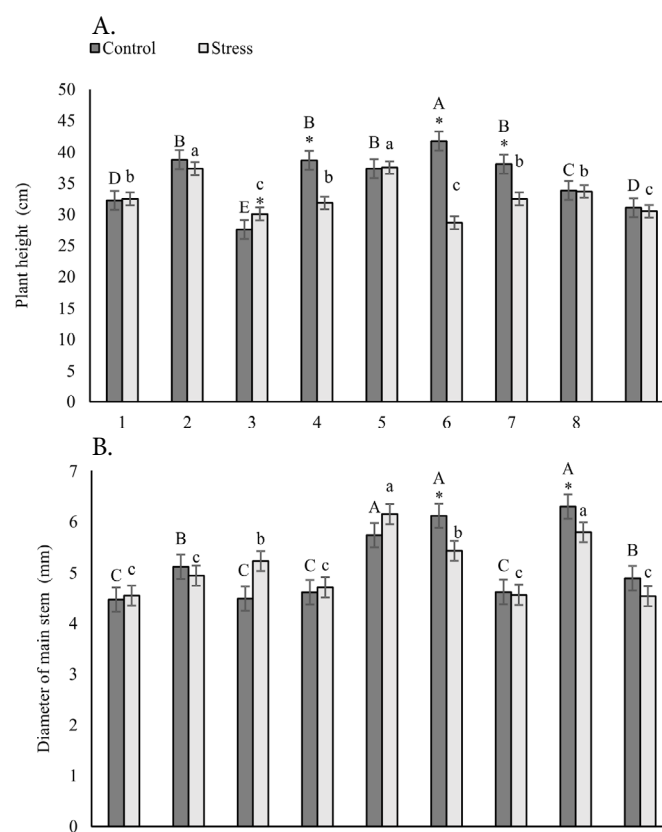
SV	DF	Mean square			
		PH	DMS	NN	NL
Cultivar (C)	8	72.35**	3.07**	4.69**	7.76**
Salinity (ECw)	1	135.98**	0.04 ^{ns}	0.12 ^{ns}	0.34 ^{ns}
C × ECw	8	47.39**	0.40**	1.15 ^{ns}	1.40 ^{ns}
Block/ ECw	6	0.69 ^{ns}	0.30 ^{ns}	0.60 ^{ns}	1.01 ^{ns}
Error	48	1.42	0.11	0.66	1.12
Mean		34.10	5.12	6.93	7.51
CV (%)		3.49	6.70	11.80	14.13

*, ** - Significant by the F test at p ≤ 0.05 and p ≤ 0.01, respectively; ns - Not significant; SV - Source of variation; DF - Degree of freedom; CV - Coefficient of variation; PH - Plant height; DMS - Diameter of the main stem; NN - Number of nodes on the main stem; NL - Number of leaves

(PH) and diameter of the main stem (DMS), indicating that cultivars presented different behaviors due to salt treatment imposed.

For the phenotypic aspects, the plant disturbances of stressed plants were demonstrated through deformations and thickening of leaves, and growth reduction in some cultivars, reflected in plant height, as seen in FM 966, CNPA 7MH, CNPA 5M, and CNPA ITA 90, as well as a reduction in diameter of the main stem, which was more visible in CNPA 5M and FMT 705 (Figure 1).

The reduction in height of plants grown in the saline environment is one of the first phenotypically visible symptoms



* Indicates statistical differences between treatments (ECw). Upper and lower case letters compare cultivars under the control and stressed conditions, respectively. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA 5M, 7 - CNPA ITA 90, 8 - FMT 705, and 9 - BRS RUBI. The vertical bar represent standard error (n = 4)

Figure 1. Plant height (A) and diameter (B) of the main stem of cotton cultivars subjected to 35 days of saline stress (6.0 dS m⁻¹)

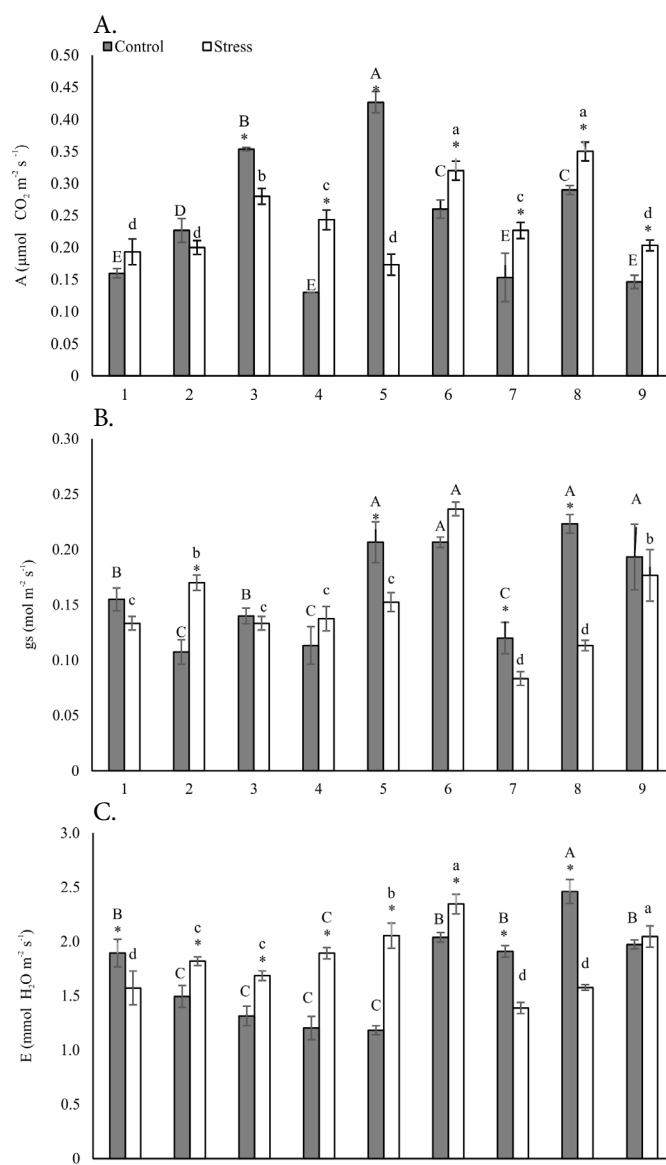
due to osmotic damage or ion toxicity from the accumulation of salts in the leaves that affect turgor and cell expansion (Acosta-Motos et al., 2017). Tolerant genotypes also show symptoms, but respond more moderately, adopting other strategies to avoid stress damage, such as redirecting energy to maintain biochemical and physiological activities, and compartmentalizing and excluding Na⁺ ions (Zhang et al., 2014)

In general, plant growth reduction was more notable in the late-cycle CNPA 5M, a Marie galant type widely tolerant of semiarid environments. As it is an arboreum cultivar, the effects of salt stress were noticed earlier than in other herbaceous cotton cultivars, with a reduction in plant height and stem diameter of 33 and 13%, respectively (Figure 1). However, these traits alone do not indicate salinity tolerance in cotton crops. Other factors should be considered, such as the stress duration, ECw, germplasm variability, and cell physiology.

The ECw adopted in this study is considered high and was based on an assay performed by Silva et al. (2017), who subjected a precocious cotton (cv. Topázio) to different salinity levels over 108 days, and found a reduction in growth traits at 6.0 dS m⁻¹, mainly in stem diameter, height, and leaf number and area.

It was found that the plants were not highly influenced by salt treatment, possibly because the period studied (35 days stress) was insufficient to cause substantial damage, at least in terms of the phenotypical aspects. However, statistical differences (p < 0.01) were identified by ANOVA for most physiological traits in the cultivars, but only for EiUA, F_o, and F_v/F_m in the treatments (ECw) (Table 3). The effect of C × ECw was determined for most traits, except Ci, indicating that cultivars responded differently to treatments, even over a short period of stress. In general, the cultivars used different mechanisms to manage saline stress: BRS Seridó, CNPA ITA 90, FMT 705, and BRS RUBI were able to overcome the osmotic stress by increasing or maintaining the A rate (Figure 2A), along with the maintenance or reduction of gs (Figure 2B), and E rates (Figure 2C). In contrast, CNPA ITA 90 and FMT 705 showed an unusual behavior by increasing the A rate even when stomata were closed (Figure 2B), to avoid the loss of water in tissues. The transpiration rate of these cultivars was reduced by 27 and 36%, respectively, indicating a reasonable adjustment in water retention. In the case of BRS Seridó, the retention rate was 17% (Figure 2C).

Tolerance to salinity was also studied by Braz et al. (2019), who used seven cotton cultivars in phase V3 and identified



* Indicates a statistical difference between treatments (ECw). Upper and lower case letters compare cultivars under the control and stressed conditions, respectively. 1 – BRS Seridó, 2 – BRS 286, 3 – FM 966, 4 – CNPA 7MH, 5 – FMT 701, 6 – CNPA 5M, 7 – CNPA ITA 90, 8 – FMT 705, and 9 – BRS RUBI. Standard error results from using four means

Figure 2. Gas exchange in cotton cultivars subjected to salt stress (ECw: 6.0 dS m⁻¹). Net photosynthesis – A (A), stomata conductance – gs (B), transpiration - E (C)

that when subjected to a more intense saline condition than those presented in this study (95 mM of NaCl), BRS Seridó, BRS 416, and 7MH cultivars physiologically adjusted by reducing the stomatal opening (gs) and transpiration, and maintaining EiUA levels, indicating regulation in the

Table 3. Synthesis of ANOVA for physiological traits in cotton cultivars subjected to salt stress

SV	DF	A	gs	E	Ci	EiUA	EiC	Fo	Fm	Fv	Fv/Fm
Cultivar (C)	8	0.03**	0.010**	0.47**	12.6 ^{ns}	0.018**	0.01**	195**	256**	266**	0.001**
Salinity ECw	1	0.001 ^{ns}	0.003 ^{ns}	0.18 ^{ns}	3.40 ^{ns}	0.004*	0.01 ^{ns}	127*	934 ^{ns}	175 ^{ns}	0.001**
C × ECw	8	0.024**	0.005**	0.66**	9.96 ^{ns}	0.027**	0.01**	40.1**	247**	236**	0.001**
Block/T	6	0.001	0.001	0.04	18.9	0.001	0.01	19.4	483	393	0.001
Error	4	0.001	0.001	0.03	8.93	0.001	0.01	9.60	269	270	0.001
Mean		0.24	0.15	1.77	326	0.14	0.01	137	558	421	0.75
CV (%)		12.6	15.0	9.38	0.91	18.3	10.0	2.25	2.93	3.90	1.19

*, ** - Significant at p ≤ 0.05 and 0.01, respectively, F test; ns - Not significant; SV - Source of variation; DF - Degree of freedom; CV - Coefficient of variation; A - Photosynthesis rate; gs - Stomatal conductance; E - Transpiration; Ci - Concentration of internal CO₂; F_o - Initial fluorescence; F_m - Maximum fluorescence; F_v - Variable fluorescence; F_v/F_m - Potential quantum yield

expression of aquaporin transcripts from roots that contribute to tolerance to osmotic stresses.

The effect of salt via irrigation water on cotton cultivars was also observed by Soares et al. (2018), who subjected BRS Topázio, BRS Rubi, and BRS Safira to different irrigation management method at ECw from 0.8 to 9.0 dS m⁻¹ in the vegetative and reproductive phases. They noted a reduction in the stomatal opening, transpiration, and internal carbon concentration, and increased values for EiC when irrigated in the vegetative and fruiting phases, indicating that salinity affects cotton crop regardless of the phase and concentration applied, thereby reflecting production losses.

The results of EiUA and EiC are shown in Figures 3A and 3B, which estimate the efficiency of water use and carboxylation, respectively. Cultivars BRS Seridó, CNPA ITA 90, FM 705, and BRS Rubi were able to better adjust the use of available water and carbon, based on the condition of salt stress adopted in this study. In contrast, CNPA 5M and CNPA 7MH, both grown in the Brazilian semiarid region, showed increased A and E rates (Figure 2C) when stressed, but maintained the EiUA and increased the EiC. Despite the loss of water, these cultivars managed to adjust osmotically, fixing the available carbon found in the substomatic chambers and directing them efficiently to the metabolic processes that follow in the production of photoassimilates. The ability to adjust osmotically was also documented by Rodrigues et al. (2016),

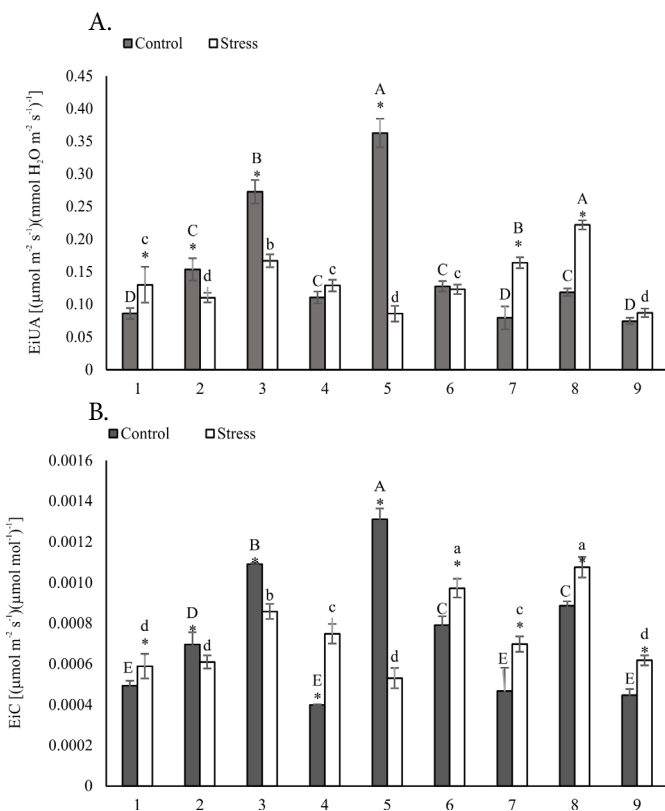
who subjected these cultivars to seven days of water stress in the R1 phase. According to the literature, the adaptation of CNPA 5M and CNPA 7MH to adverse environmental conditions relates to the genetic basis of both cultivars, which contain genes from the latifolium and Marie galant subspecies that provide broad tolerance to adverse climate and soil conditions in the semiarid region (Crisóstomo & Freire, 1982; Carvalho et al., 2014).

The fluorescence values of the cultivars subjected to salt treatment are shown in Figure 4. This is consistent with results obtained from gas exchanges, mainly in BRS Seridó, CNPA ITA 90, and FM 705, which maintained the photosynthetic efficiency during stress treatment (Figures 4A, B, and C), indicating that salinity at 6.0 dS m⁻¹ may not have affected the transfer of electrons of the antenna complex to photosystem II. According to the literature, the maintenance of photosynthetic efficiency is a valuable indicator of tolerance to abiotic stress. The Fv/Fm ratio in the range of 0.75 and 0.85 is highly correlated with the quantum yield of net photosynthesis of intact leaves exposed to various levels of photo-inhibition (Demmig & Bjorkman, 1987). A reduction in Fv/Fm means photo-inhibitory damage in plants subjected to environmental stresses, although it is important to distinguish increases in F₀ from decreases in the quantity of light fluorescence. An increase in F₀, as seen in F_m 966 and CNPA 7MH, is characteristic of the destruction of PSII reaction centers, whereas a decline, such as in FMT 701, may indicate an increase in non-photochemical quenching; photo-inhibition produces both of these changes (Bolhar-Nordenkamp et al., 1989).

The set of physiological traits adopted here offer a broad contribution to the selection of plants that are tolerant to environmental stresses. Despite the value of each trait, the estimate of photosynthesis rate is relevant because it contributes to identifying genotypes tolerant to salt stress.

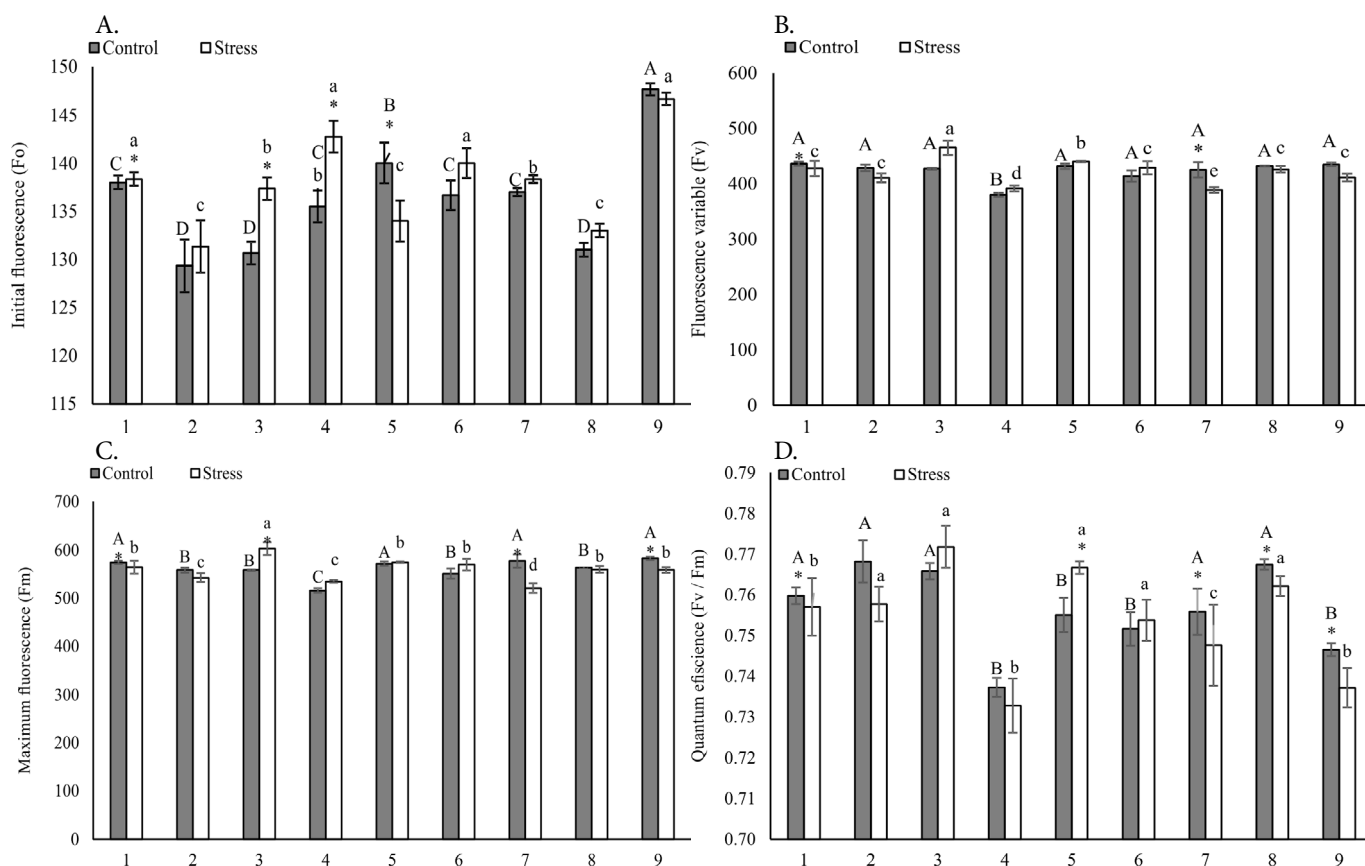
In general, the growth of plants under salt stress is accompanied by a strong reduction in photosynthesis rate and chlorophyll content, the impact of which depends on the level of germplasm tolerance. Since chlorophyll content is directly correlated with active plant growth, this decrease leads to substantial damage to the photosynthetic mechanism, particularly in sensitive species (Meloni et al., 2003; Lee et al., 2013; Zhang et al., 2014). Zhang et al. (2014) studied the effects of salinity on the growth, physiological, and biochemical traits in salt-tolerant (CCRI-79) and sensitive (Simian 3) cotton cultivars subjected to 80 to 240 mM NaCl, and found a wide reduction in growth, net photosynthetic rate, Fv/Fm ratio, and stomatal conductance in sensitive plants under seven days of salt stress. The physiological and biochemical profiles of CCRI-79 demonstrated an effective protection mechanism, including mitigation of oxidative stress and lipid peroxidation during the period of stress.

Although biochemical criteria were not adopted for evaluation, the cultivars BRS Seridó, CNPA ITA 90, and FM 705 were considered tolerant to salt stress based on the results of gas exchange and fluorescence observed under the experimental conditions.



* Indicates a statistical difference between treatments (ECw). Upper and lower case letters compare cultivars under the control and stressed conditions, respectively. 1 - BRS Seridó, 2 - BRS 286, 3 - FM 966, 4 - CNPA 7MH, 5 - FMT 701, 6 - CNPA 5M, 7 - CNPA ITA 90, 8 - FMT 705, and 9 - BRS RUBI. Standard error results from using four means

Figure 3. Instantaneous water use efficiency – EiUA (A) and intrinsic efficiency of carboxylation – EiC (B) in cotton cultivars subjected to salt stress (ECw: 6.0 dS m⁻¹)

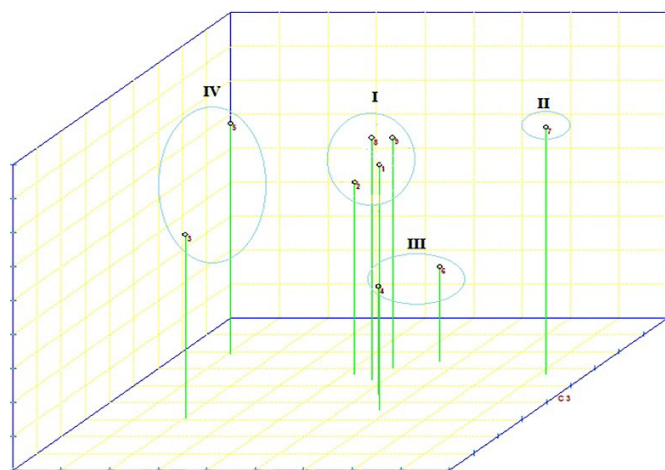


* Indicates a statistical difference between treatments (ECw). Upper and lower case letters compare cultivars under the control and stressed conditions, respectively. 1 – BRS Seridó, 2 – BRS 286, 3 – FM 966, 4 – CNPA 7MH, 5 – FMT 701, 6 – CNPA 5M, 7 – CNPA ITA 90, 8 – FMT 705, 9 – BRS RUBI. The vertical bars represent the standard error (n = 4)

Figure 4. Fluorescence traits in cotton cultivars subjected to salt stress (ECw: 6.0 dS m⁻¹)

Non-hierarchical (PCA) and hierarchical (UPGMA) clustering methods were used to classify cotton cultivars under salt stress. The relative contribution of traits to genetic divergence was estimated as described by Singh (1981).

Table 4 shows the eigenvalue estimates and cumulative percentages of the main components. The variation was explained by the first three eigenvalues (82.19%), with the graphic dispersion shown in Figure 5. Four classification groups were identified: GI – represented by BRS Seridó, BRS 286, FMT 705, and BRS Rubi; GII – containing only CNPA ITA 90, GIII – grouping CNPA 5M, and CNPA 7MH, and G4 – clustering FM 966, and FMT 701. The relative contribution of the traits, based on Singh (1981), showed that A, E, and gs contributed widely to the classification of cultivars, with values of 38, 28, and 21%, respectively. The composition of these groups (Figure 5) was also confirmed in the dendrogram obtained via UPGMA (Figure 6), indicating coherence of both



1 – BRS Seridó, 2 – BRS 286, 3 – FM 966, 4 – CNPA 7MH, 5 – FMT 701, 6 – CNPA 5M, 7 – CNPA ITA 90, 8 – FMT 705, and 9 – BRS RUBI

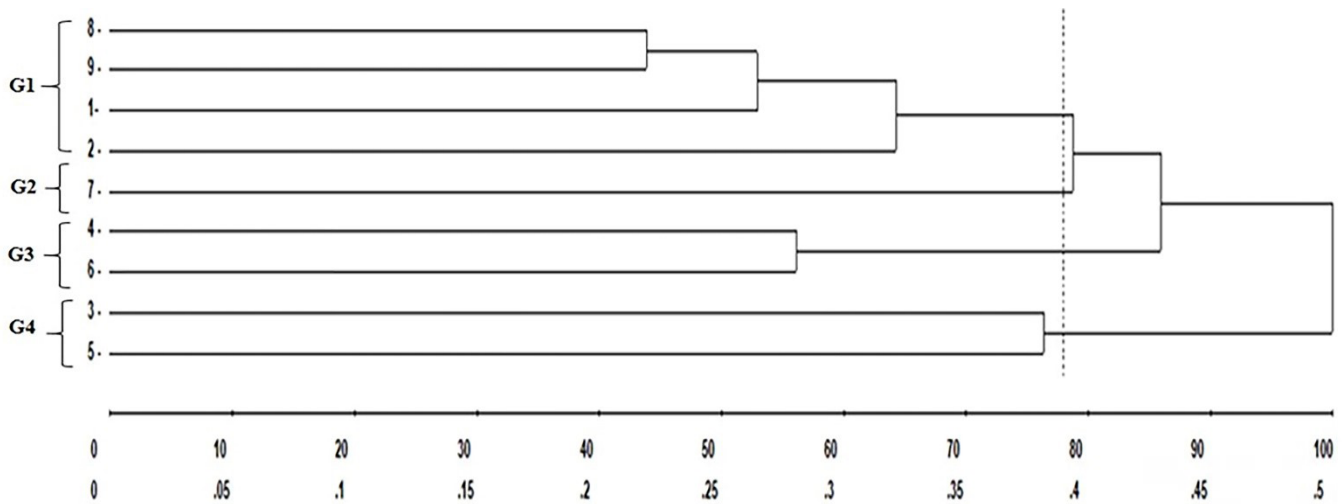
Figure 5. Graphical dispersion of nine cotton cultivars subjected to salt stress

Table 4. Eigenvalues, variance, and accumulated variance (AV) of principal components (PC) obtained from matrix performed with growth and physiological traits of cotton cultivars under salt stress

PC	Eigenvalues	Variance (%)	AV
PC 1	4.360	39.64	39.64
PC 2	3.004	27.31	66.95
PC 3	1.676	15.23	82.19
PC 4	.8545	7.768	89.96
PC 5	.6274	5.704	95.67
PC 6	.3893	3.543	99.21
PC 7	.0717	.6624	99.86
PC 8	.0151	.1373	100.0

methodologies for the classification of different cotton cultivars using growth and physiological traits.

Considering the contribution of these results to breeding programs focused on saline environments, the use of cultivars from groups G1 and G5 provides prospects for obtaining progenies with the potential for genetic tolerance to saline stress and broad adaptation to semiarid and cerrado environments. As they are all commercial cultivars, there is an additional advantage of maintaining the other fiber and yarn characteristics, making this improvement simpler for selection procedures.



1 – BRS Seridó, 2 – BRS 286, 3 – FM 966, 4 – CNPA 7MH, 5 – FMT 701, 6 – CNPA 5M, 7 – CNPA ITA 90, 8 – FMT 705, and 9 – BRS RUBI

Figure 6. Dendrogram obtained by UPGMA, from similarity matrix obtained from nine cotton cultivars subjected to salt stress

CONCLUSIONS

1. The clustering obtained from the UPGMA and PC multivariate methods was consistent for the classification of cultivars. The cultivars BRS Seridó, BRS 286, FMT 705, and BRS Rubi, which were tolerant to salt stress, were clustered in the same group.

2. Photosynthesis, transpiration, and stomatal conductance data were the main contributors to the classification of cultivars with the principal component method.

LITERATURE CITED

- Abul-Naas, A. A.; Omran, M. S. Salt tolerance of seventeen cotton cultivars during germination and early seedling development. *Zucker Pflanzenbau* v.140, p.229-236, 1975.
- Acosta-Motos, J. R.; Ortuño, M. F.; Bernal-Vicente, A.; Diaz-Vivancos P.; Sanchez-Blanco, M. J.; Hernandez, J. A. Plant responses to salt stress: Adaptive mechanisms. *Agronomy*, v.7, p.1-38, 2017. <https://doi.org/10.3390/agronomy7010018>
- Ayers, R. S.; Westcot, D. W. *A Qualidade de água na agricultura* 2.ed. Campina Grande: UFPB, 1999. 153p.
- Bolhar-Nordenkamp, H. R.; Long, S. P.; Baker, N. R.; Oquist, G.; Schreiber, U.; Lechner, E. G. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Functional Ecology*, v.3, p.497-514, 1989. <https://doi.org/10.2307/2389624>
- Braz, C. C. L.; Fernandes, P. D.; Barbosa, D. D.; Dutra, W. F.; Silva, R. C.; Lima, L. M.; Cavalcanti, J. J. V.; Farias, F. J. C.; Santos, R. C. Expression of aquaporin subtypes (GhPIP1;1, GhTIP2;1 and GhSIP1;3) in cotton (*Gossypium hirsutum*) submitted to salt stress. *AoB PLANTS*, v.11, p.1-11, 2019. <https://doi.org/10.1093/aobpla/plz072>
- Carvalho, L. P. de; Farias, F. J. C.; Lima, M. M. de A.; Rodrigues, J. I. da S. Inheritance of different fiber colors in cotton (*Gossypium barbadense* L.). *Crop Breeding and Applied Biotechnology*.v.14, p.256-260, 2014. <https://doi.org/10.1590/1984-70332014v14n4n40>
- Crisóstomo, J. R.; Freire, E. C. Origem e características das variedades de algodoeiros arbóreo e herbáceo indicados atualmente para o Nordeste. Versão preliminar. Campina Grande: Embrapa-CNPA, 1982. 18p.
- Cruz, C. D. Genes - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum Agronomia*, v.35, p.271-276, 2013.
- Cruz, C. D.; Regazzi, A. J.; Carneiro, P. C. S. *Modelos biométricos aplicados ao melhoramento genético*. 4.ed. Viçosa: UFV, 2012. 514p.
- Demmig, B.; Bjorkman, O. Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O₂ evolution in leaves of higher plants. *Planta*, v.171, p.171-184, 1987. <https://doi.org/10.1007/BF00391092>
- Dutra, W. F.; Guerra, Y. L.; Ramos, J. P. C.; Fernandes, P. D.; Silva, C. R. C.; Bertioli, D. J.; Leal-Bertioli, S. C. M.; Santos, R. C. Introgression of wild alleles into the tetraploid peanut crop to improve water use efficiency, earliness and yield. *Plos One*. v.13, p.1-15, 2018. <https://doi.org/10.1371/journal.pone.0198776>
- Härdle, W.; Simar, L. *Applied multivariate statistical analysis*. Berlin: MD Tech, 2003. 488p. <https://doi.org/10.1007/978-3-662-05802-2>
- Jesus, J. M.; Danko, A. S.; Fiúza, A.; Borges, M. T. Phytoremediation of salt-affected soils: a review of processes, applicability, and the impact of climate change. *Environmental Science and Pollution Research*, v.22, p.6511-6525, 2015. <https://doi.org/10.1007/s11356-015-4205-4>
- Lee, M. H.; Cho, E. J.; Wi, S. G.; Bae, H.; Kim, J. E.; Cho, J. Y.; Lee, S.; Kim, J. H.; Chung, B. Y. Divergences in morphological changes and antioxidant responses in salt-tolerant and salt-sensitive rice seedlings after salt stress. *Plant Physiology Biochemistry*, v.70, p.325-335, 2013. <https://doi.org/10.1016/j.plaphy.2013.05.047>
- Marur, C. J.; Ruano, O. A reference system for determination of cotton plant development. *Revista de Oleaginosas e Fibras*, v.5, p.313-317, 2001.
- Medeiros, J. F. de. *Qualidade de água de irrigação e evolução da salinidade nas propriedades assistidas pelo GAT nos Estados de RN, PB e CE*. Campina Grande: UFPB, 1992. 192p. Dissertação Mestrado
- Meloni, D. A.; Oliva, M. A.; Martinez, C. A.; Cambraia, J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany*, v.49, p.69-76, 2003. [https://doi.org/10.1016/S0098-8472\(02\)00058-8](https://doi.org/10.1016/S0098-8472(02)00058-8)

- Munns, R.; Gilliam, M. Salinity tolerance of crops - what is the cost? *New Phytologist*, v.208, p.668-673, 2015. <https://doi.org/10.1111/nph.13519>
- Novais, R. F.; Neves, J. C. L.; Barros, N. F. Ensaio em ambiente controlado. In: Oliveira, A. J.; Garrido, W. E.; Araújo, J. D.; Lourenço, S. Métodos de pesquisa em fertilidade do solo. Brasília, DF: Embrapa, 1991. Cap. 2, p.189-198.
- Ramos, J. P. C.; Luz, L. N. da; Cavalcanti, J. J. V.; Lima, L. M. de; Freire, R. M. M.; Melo Filho, P. de A.; Santos R. C. dos. Clustering fastigiata peanut accessions for selection of early-mature types suitable for the food market. *Australian Journal of Crop Science*, v.9, p.1089-1094, 2015.
- Resende, M. D. V. de. Matemática e estatística na análise de experimentos e no melhoramento genético. Colombo: Embrapa Florestas, 2007. 561p.
- Rhoades, J. D.; Kandiah, A.; Mashali, A. M. The use of saline waters for crop production. *Pimprenta: Rome Serie: FAO. Irrigation and drainage*, v.48, 1992. 133p.
- Rodrigues, J. D.; Silva, C. R. C. da; Pereira, R. F.; Ramos, J. P. C.; Melo Filho, P. de A.; Cavalcanti, J. J. V.; Santos, R. C. Characterization of water-stress tolerant cotton cultivars based on plant growth and in activity of antioxidant enzymes. *African Journal of Agricultural Research*, v.11, p.3763-3770, 2016. <https://doi.org/10.5897/AJAR2016.11301>
- Salassier, B.; Soares, A.A.; Mantovani, E.C. Manual de irrigação, 8. ed. Viçosa: UFV, 2006. 625p
- Silva, A. A. R. da; Lima, G. S. de; Azevedo, C. A. V. de; Soares, L. A. dos A.; Gheyi, H. R.; Oliveira, R. C. de. Potassium fertilization in the cultivation of colored cotton irrigated with saline water. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v.21, p.628-633, 2017. <https://doi.org/10.1590/1807-1929/agriambi.v21n9p628-633>
- Singh, D. The relative importance of characters affecting genetic divergence. *Indian Journal of Genetics and Plant Breeding*, v.41, p.237-245, 1981
- Soares, L. A. dos A. Fernandes, P. D., Lima, G. S. de; Suassuna, J. F.; Pereira, R. F. Gas exchanges and production of colored cotton irrigated with saline water at different phenological stages. *Revista de Ciências Agronômica*, v.49, p.1-10, 2018. <https://doi.org/10.5935/1806-6690.20180027>
- Sokal, R. R.; Rohlf, F. J. The comparison of dendrograms by objective methods. *International Association for Plant Taxonomy*, v.11, p.33-40, 1962. <https://doi.org/10.2307/1217208>
- Zhang, L.; Ma, H.; Chen, T.; Pen, J.; Yu, S.; Zhao, X. Morphological and physiological responses of cotton (*Gossypium hirsutum* L.) plants to salinity. *PLoS One*, v.9, p.1-14, 2014. <https://doi.org/10.1371/journal.pone.0112807>