Irrigation Science
https://doi.org/10.1007/s00271-020-00682-3

ORIGINAL PAPER

Saline water irrigation strategies in two production cycles of naturally colored cotton

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Received: 19 March 2019 / Accepted: 22 June 2020

Abstract
Scarcity of good quality water is a limiting factor for irrigated agriculture, especially in a semi-arid region, which induces the use of waters with high salt levels to irrigate crops. As a result, it becomes necessary to use salt tolerant genotypes and appropriate cultivation strategies that enable their production. With this focus, this study was carried out to evaluate the production components of three naturally colored cotton genotypes ('BRS Rubi', 'BRS Topázio', and 'BRS Safira'), subjected to various irrigation management strategies, varying the stages of development in which plants were irrigated with saline water. The study also aimed to evaluate the influence of the water salinity level at which seeds were formed, in a new production cycle, using the same irrigation strategies and varying the phenological stages. There were seven salinity management strategies in the first year of cultivation and ten in the second year, both experiments were conducted in a randomized block design. Among the genotypes, 'BRS Rubi' was the most tolerant to salinity, with respect to the intrinsic characteristics of the fiber, regardless of the development stage. In the second production cycle, plants grown from seeds formed under salt stress during the stages of flowering and yield formation had increases in lint weight and fiber quality. In the second year, the oil content of the cotton genotypes was not compromised by the cumulative salt stress, considering seeds produced in plants irrigated with saline water in the previous cycle.

Introduction

Scarcity of water resources in arid and semi-arid regions involves quantitative and qualitative aspects, causing restrictions on the use for human consumption, animal consumption, and irrigation. These regions commonly have water sources with high concentration of salts, mainly sodium chloride, limiting their use in agriculture (Mermoud et al. 2005; Khan et al. 2006). Sodium and chloride were the dominant ions in the crystalline basement of the Brazilian Northeast regardless of location, salinity level, or place of origin (Silva Junior et al. 1999). However, in many areas, irrigation with saline water has been increasingly necessary, due to limited fresh water resources and high evapotranspiration demands of cotton in the region. Such use generally causes limitations on plant growth and yield due to the reduction of the osmotic potential in the soil solution and may also cause ionic effects, such as toxicity and nutritional imbalance (Aydin et al. 2012; Mguis et al. 2012).

Saline water can be used to irrigate crops in development stage(s) in which its effect on the reduction of production is lower (Ashraf and Harris 2004), depending on the degree of tolerance of the species to salinity and on improvements in water and soil management practices (Lacerda et al. 2003). Cotton (*Gossypium hirsutum* L.) is among the species suitable for cultivation under saline conditions, because of its socio-economic importance for agribusiness and because it is tolerant to salt stress and has a relatively low daily water consumption (Paiva et al. 2013).

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Published online: 30 June 2020

According to the classification described in Ayers and Westcot (1999), white fiber cotton is considered tolerant to salt stress, with a salinity threshold of 7.7 dS m^{-1} in the soil saturation extract; with reductions of 10, 25, 50, and 100% in its yield at electrical conductivity levels of 9.6, 13.0, 17.0 and 27.0 dS m^{-1}, respectively (Maas and Hoffman 1977). However, the use of this classification cannot be generalized, considering the development of new genotypes, including those of naturally colored cotton, and the tolerance to salinity varies among the developmental stages of the crop (Maas 1990).

Despite this tolerance, several researchers have reported negative effects of salinity on naturally colored cotton plants. In general, among its effects, salinity slows and reduces germination and emergence, slows branch growth and affects several other growth components; it also compromises some characteristics of fiber quality, when the plants are irrigated with high salinity waters (Lima et al. 2017; Silva et al. 2017).

Although studies on cotton tolerance to salt stress have already been carried out, research should be intensified to identify the phenological phases in which the crop is more tolerant or sensitive to salinity, considering the release of new genetic materials, due to their importance for adoption of appropriate agronomic strategies for agricultural production in saline soils, or even under conditions where only water with higher salt content is available (Lauchli and Epstein 1990). The reference values of salt tolerance at each developmental stage and plant recovery in the phenological phases following salt stress, as well as the consequences of the cumulative effect of saline stress in successive cultivation cycles need to be properly investigated.

Considering these aspects and the fact that there are no reports of similar research with cotton plants, this study aimed to evaluate the production components of naturally colored cotton genotypes, subjected to irrigation management strategies, varying the development stages in which the plants were irrigated with saline water, as well as to assess the influence of the salinity level at which the seeds were formed, in a new productive cycle, using the same strategies of irrigation and variation of the phenological stages.

Materials and methods

Two experiments were conducted in protected environment, due to the nature of the studied factors, at the Center of Technology and Natural Resources–CTRN of the Federal University of Campina Grande—UFCG, located in the municipality of Campina Grande, Paraíba, Brazil, at geographic coordinates 07°15′18″ S, 35°52′28″ W and mean altitude of 550 m. The climate of the region is Bsh, which represents a semi-arid, hot, and dry climate according to Köppen's climate classification, adapted to Brazil (Coelho and Soncin 1982).

Table 1 Management strategies of irrigation with low and high-salinity water in the vegetative (16–37 DAS), flowering (37–59 DAS) and yield formation (59–113 DAS) phenological stages of the cotton genotypes

Management strategies of irrigation	Phenological stages			
	Vegetative (A)	Flowering (B)	Yield formation (C)	Total
	dS m^{-1}			
$A_1B_1C_1$	0.8	0.8	0.8	2.4
$A_2B_1C_1$	9.0	0.8	0.8	10.6
$A_1B_2C_1$	0.8	9.0	0.8	10.6
$A_1B_1C_2$	0.8	0.8	9.0	10.6
$A_2B_1C_2$	9.0	0.8	9.0	18.8
$A_2B_2C_1$	9.0	9.0	0.8	18.8
$A_1B_2C_2$	0.8	9.0	9.0	18.8

Index 1 (A_1, B_1, C_1): low salinity (0.8 dS m^{-1}); Index 2 (A_2, B_2, C_2): high salinity (9 dS m^{-1}), at each growth stage

Both experiments used three genotypes of naturally colored cotton ('BRS Rubi'; 'BRS Safira', and 'BRS Topázio') subjected to different management strategies of irrigation with saline water, varying according to the phenological stages of the plants: vegetative (A)—from emergence of first true leaf to anthesis of first flower; flowering (B)—from anthesis of first flower to opening of first boll; yield formation (C)—opening of first boll to final harvest of the bolls in the first cycle.

The herbaceous cotton genotype BRS Rubi has dark brown or reddish brown fiber, and a cultivation cycle of 120–140 days and average yield of 1539 kg ha^{-1} under rainfed regime in the Northeast region. BRS Topázio is a light brown fiber genotype, with a high percentage of fiber (43.5%), high uniformity (85.2%) and strength (313 m kg^{-1}), and an average yield of 2825 kg ha^{-1} under irrigation, in addition to having a short cycle, 115 days. The BRS Safira genotype has a dark brown or reddish brown fiber, but in a lighter shade than the BRS Rubi fiber. It has a cultivation cycle between 120 and 140 days. Under rainfed conditions, in the Northeast region, it can produce up to 3000 kg ha^{-1} (EMBRAPA 2011).

Experiment I: strategies of saline water irrigation management varying the development stages of the cotton genotypes

Cotton genotypes were subjected to seven irrigation management strategies, receiving water with two levels of electrical conductivity (ECw), low salinity (ECw = 0.8 dS m^{-1}) and high salinity (9 dS m^{-1}), varying the phenological stage, to form the following treatments (Table 1): 1—$A_1B_1C_1$—plants irrigated with low-salinity water along the entire cycle,

identified by the index 1 in the phenological stages; 2—$A_2B_1C_1$—plants under salt stress during the vegetative stage (index 2 in stage A), receiving 9 dS m^{-1} water from 16 days after sowing (DAS) until the beginning of flowering, which occurred at 37 DAS, and then low-salinity water until the end of the cycle; 3—$A_1B_2C_1$—plants under salt stress during the flowering stage (irrigation with high-salinity water from 37 DAS until yield formation, at 59 DAS) and irrigated with low-salinity water at the other stages; 4—$A_1B_1C_2$—plants irrigated with good quality water during the vegetative and flowering stages and high-salinity water from 59 DAS, until the end of the cycle, corresponding to the yield formation stage; 5—$A_2B_1C_2$—plants irrigated with high-salinity water during the vegetative and yield formation stages, receiving non-saline water during the flowering stage; 6—$A_2B_2C_1$—irrigation with high salinity during the vegetative and flowering stages and low-salinity water during the period of yield formation; 7—$A_1B_2C_2$—irrigation with low-salinity water during the vegetative stage and high-salinity water during the stages of flowering and yield formation.

The experimental design was randomized blocks in a 3 × 7 factorial scheme (3 genotypes and 7 irrigation management strategies), resulting in 21 treatments, with 3 replicates and three plants per plot, totaling 189 plants.

Experiment II: strategies of irrigation with high-salinity water combining phenological stages of two consecutive cycles for plants grown from seeds produced under salt stress in the first cycle

In the second experiment, the irrigation management strategies resulted from the combination of phenological stages between both cycles, using high-salinity water alternated with low-salinity water, thus characterized as cumulative salt stress. The following letters were used for identification: A—plants grown from seeds formed when low-salinity water (0.8 dS m^{-1}) was applied along the entire previous cycle (1—$A_1B_1C_1$); B—plants grown from seeds formed in the first cycle with saline water (9 dS m^{-1}) in the flowering stage (3—$A_1B_2C_1$) and good quality water in the others; C—plants grown from seeds formed when saline water was applied only in the yield formation stage (4—$A_1B_1C_2$); BC—plants grown from seeds formed in the previous cycle with high-salinity water in the stages of flowering and yield formation (7—$A_1B_2C_2$). The ten treatments forming the irrigation strategies in the new experiment are detailed in Table 2.

The vegetative stage corresponded to the period between emergence of first true leaf and opening of first flower (20–55 DAS); flowering: opening of first flower until opening of first boll (55–80 DAS); yield formation: opening of first boll until final harvest of the bolls (80–105 DAS). The experimental design was randomized blocks, with treatments arranged in a 3 × 10 factorial scheme, which corresponded to three cotton genotypes and ten irrigation management strategies. Each treatment was replicated, three times, each replicate consisting of two plants per plot for a total of 180 plants.

Experimental procedures common to both experiments

Plants were grown in 20 L plastic pots (35 cm height × 31 cm upper diameter × 20 cm lower diameter), covered at the bottom with polyester geotextile with grammage of 130 g m^{-2}

Table 2 Strategies of salinity management in different phenological stages of cotton in the second experiment, with information on the treatments from which the seeds were collected in the first cycle

Management strategies of irrigation		Phenological stages			
		Vegetative (A)	Flowering (B)	Yield formation (C)	Year 2 total
Year 1 treatment	Year 2 treatment	dS m^{-1}			
$A_1B_1C_1$	A-S0	0.8	0.8	0.8	2.4
$A_2B_1C_1$	B-S0	0.8	0.8	0.8	2.4
	B-VS	9.0	0.8	0.8	10.6
	B-FLS	0.8	9.0	0.8	10.6
$A_1B_2C_1$	C-S0	0.8	0.8	0.8	2.4
	C-VS	9.0	0.8	0.8	10.6
	C-YFS	0.8	0.8	9.0	10.6
$A_1B_2C_2$	BC-S0	0.8	0.8	0.8	2.4
	BC-VS	9.0	0.8	0.8	10.6
	BC-FFS	0.8	9.0	9.0	18.8

Experiment II: S0: low-salinity water applied along the entire previous cycle; VS, FLS, YFS and FFS: high-salinity water in the vegetative stage, flowering stage, yield formation stage and stages of flowering and yield formation, respectively

to avoid loss of soil material, and filled with a 3 cm layer of crushed stone. The bottom of each pot was connected to a plastic transparent tube that led to a 2.0 L container to collect the drained water. Then, the pots received 24.5 kg of soil material from a eutrophic Regolithic Neosol (Psamment) according to Benedetti (2011), with sandy loam texture (collected in the 0–20 cm layer), which was previously pounded to breakup clods and sieved with 5 mm mesh. Its physical and chemical attributes, determined in the laboratory, before the sowing of both experiments, are presented in Table 3.

Before sowing, 500 g of organic matter (earthworm humus) was incorporated in the soil material to improve soil structure and moisture retention. To meet nutritional requirements, plants were fertilized with N, P and K, according to the recommendation of fertilization for pot experiments described in Novais et al. (1991); i.e. applying 100, 300 and 150 mg kg^{-1} of soil of N, P_2O_5 and K_2O, respectively, in the forms of ammonium sulfate, single superphosphate and potassium chloride.

The entire recommendation of P was applied at planting, along with only 1/3 of the recommendations of N and K. The remaining 2/3 of N and K were applied as topdressing, through irrigation water, at 45 and 65 days after sowing (DAS) in equal parts. The pots were arranged in single rows spaced by 1 m and plants were spaced by 0.6 m in each row. To enhance plant nutrition and meet possible deficiencies of micronutrients, foliar fertilization was applied during early flowering, at 45 DAS in the first experiment and at 57 DAS in the second experiment, using 3 L of solution containing 2.5 g L^{-1} of Ubyfol® (N—15%; P_2O_5—15%; K_2O—15%; Ca—1%; Mg—1.4%; S—2.7%; Zn—0.5%; B—0.05%; Fe—0.5%; Mn—0.05%; Cu—0.5% and Mo—0.02%).

For the first experiment, the seeds of the three cotton genotypes were provided by Embrapa Cotton and five seeds were planted in each pot, at 3 cm depth, equidistantly distributed. After germination, thinning was performed to leave only the most vigorous seedling in each pot. Soil water content was kept at a level equivalent to the maximum retention capacity (FC) in all experimental units, using low-salinity water (0.8 dS m^{-1}), until the appearance of the first true leaf, when treatments began to be applied. Irrigations were carried out every day at 17:00 h, applying in each pot the water volume corresponding to the water requirement of the plant. The volume applied in each irrigation event was estimated by water balance, based on the terms of Eq. 1, where: *WC* is water consumption, considering the water volume applied to the plants (*Va*) on the previous day; Vd is the volume drained, quantified in the morning of the next day, and *LF* is the desired leaching fraction, estimated at 0.20, to partially reduce the accumulation of salts from the irrigation water in the root zone.

$$WC = \frac{Va - Vd}{1 - LF}. \quad (1)$$

The irrigation water used in the treatment of lowest salinity level (0.8 dS m^{-1}) was obtained by diluting water from the public supply system of Campina Grande-PB, using rainwater. Water with 9 dS m^{-1} (high ECw) was prepared based on the relationship between ECw (dS m^{-1}) and concentration of salts ($mmol_c\ L^{-1} = 10 \times ECw$), according to Rhoades et al. (2000).

Bolls were harvested from each plant as they reached the harvest point, the seed cotton and the lint cotton weights were quantified. The bolls of each plot were sent for analysis at the Laboratory of Fibers of Embrapa Cotton, using a HVI (High Volume Instruments) model 900 from USTER, according to NBR ISO 139: 2008 (standard for conditioning and testing) of the Brazilian Association of Technical Standards (ABNT), which are: temperature 20 ºC (±2) and humidity 65% (±2), device to obtain the following data: fiber percentage (%), fiber length (mm), fiber length uniformity (%), and short fiber index (%). Oil content in the seeds was determined at the Multidisciplinary Laboratory of Embrapa Cotton, in Campina Grande-PB, after drying and processing, with moisture corrected to 10%, in a non-destructive manner and using a nuclear magnetic resonance (NMR) spectrometer H1 Oxford MQA 7005 (American Oil Chemists' Society 2000).

For the classification of genotypes, the criterion of reduction in relative yield was adopted according to Richards (1954), with four classification levels: T (tolerant; 0–20%), MT (moderately tolerant; 20–40%), MS (moderately

Table 3 Physical and chemical attributes of the soil material used in the experiments

Density	Total porosity	Water content (%)		Available water	Exchange complex					
					Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	pH_{sp}	EC_{se}
kg dm^{-3}	%	0.33 atm	15 atm	%	$cmol_c\ kg^{-1}$				–	dS m^{-1}
Experiment I										
1.67	38.59	11.48	2.41	9.07	2.37	3.09	0.37	0.18	5.24	0.20
Experiment II										
2.71	52.03	8.40	4.64	3.36	2.10	2.57	0.06	0.14	5.80	0.22

Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^{+} and K^{+} extracted with 1 M NH_4OAc; P extracted with Mehlich-1; pH_{sp}, pH of the saturated paste and ECse electrical conductivity of the saturation extract

sensitive; 40–60%), and S (sensitive; > 60%). It was based on the percentage of loss in cotton lint mass under high saline level (9.0 dS m^{-1}), compared to the low salinity condition (0.8 dS m^{-1}) in the vegetative, flowering, yield formation and flowering/yield formation phases. These percentage losses were used as indices to compare the salt tolerance of different genetic materials.

Data were evaluated using the *F* test, and, when significant, means were compared by Scott–Knott's grouping test, at 0.05 probability level, for salinity strategies, and by Tukey's test, also at 0.05 probability level, for cotton plant genotypes. (Ferreira 2011).

Results

According to the results of the analysis of variance, there were differences in the interaction between salinity management strategies (MS) and cotton genotypes (G), with significant effect ($p < 0.01$) on lint cotton weight (LCW), fiber percentage (%Fiber), fiber length (UHM), short fiber index (SFI) and oil content (%Oil), differed fiber length uniformity (UNF), which differed only between genotypes. In the second experiment, the interaction between factors (MS x G) affected seed cotton weight, LCW, %Fiber, UHM and SFI.

Treatment T1 ($A_1B_1C_1$) had highest cotton seed weight (253.09 g per plant), followed by T2 (227.81 g) and T3 (201.71 g); and the lowest seed weights were for strategies T4, T5, T6 and T7, whose seed weights were 45.92%, 50.53%, 45.26% and 58.24%, lower than T1 (Fig. 1a). The strategy T7 resulted in the lowest seed cotton weight, demonstrating greater sensitivity of cotton when subjected to salt stress successively in the stages of flowering and yield formation. For seed cotton weight as a function of different cotton genotypes, averaged across all treatments (Fig. 1b), the genotype 'BRS Topázio' had greatest accumulation of seed cotton weight (187.36 g per plant), which was 16.78% and 11.22% higher than the values of 'BRS Rubi' and 'BRS Safira', respectively.

In the second experiment (Fig. 2), irrigation with saline water in the vegetative stage (B-VS, C-VS and BC-VS) was less significant for the genotype 'BRS Safira', resulting in reductions of 23.72%, 13.51% and 25.82% in seed cotton weight, respectively, in comparison to plants irrigated with 0.8 dS m^{-1} water. However, salt stress applied during the yield formation stage (C-YFS) caused less damage to seed cotton weight; the genotype 'BRS Topázio' had the greatest seed cotton production (154 g per plant). At the end of the second production year, seed cotton weight in the genotype 'BRS Topázio' increased by 11.13%, in comparison to plants of this same genotype irrigated using water with ECw of 9.0 dS m^{-1} during the yield formation of the first experiment (Fig. 2).

Considering the salinity management strategies studied in the first experiment (Fig. 1a, b), it is possible to see that water salinity had the smallest impact on the lint cotton weight of 'BRS Safira'. In general, irrigation with 9.0 dS m^{-1} water caused reduction in lint cotton weight, in each stage of exposure, but the lowest reduction was found when high-salinity water was applied only during the vegetative stage ($A_2B_1C_1$), with mean decrease of 7.60% compared with the non-saline treatment ($A_1B_1C_1$). Also in Experiment I, plants were more sensitive when irrigated using 9.0 dS m^{-1} water during the yield formation stage ($A_1B_1C_2$). Differently, in the second experiment (Fig. 3d, e f), plants subjected to salt stress during the vegetative stage, corresponding to the managements T3—B-VS, T6—C-VS and T9—BC-VS, were the most affected by salinity, with reductions of 40.35%, 42.35% and 44.44% in lint cotton production, respectively, compared

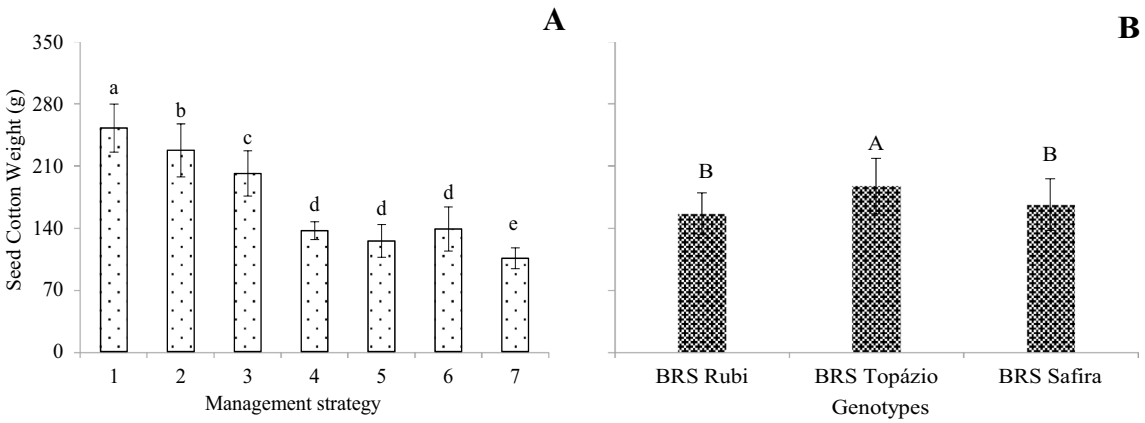

Fig. 1 Test of means for seed cotton weight as a function of salinity management strategies (**a**) and cotton genotypes (**b**) in the first experiment. Same lowercase letters indicate no significant difference between management strategies (Scott–Knott, $p < 0.05$) and uppercase between genotypes (Tukey, $p < 0.05$)

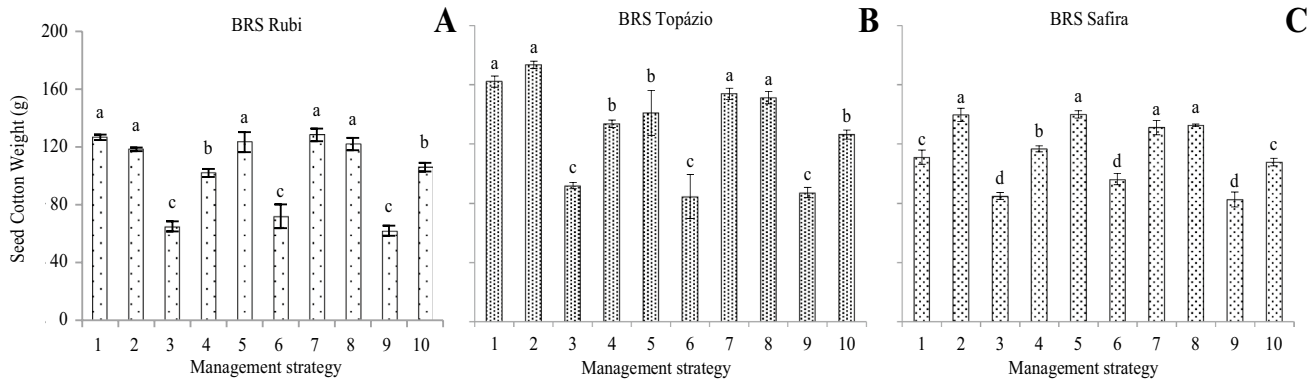

Fig. 2 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for seed cotton weight in the second experiment. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

with plants under no application of saline water (T1), stage when salt stress was applied at flowering and or yield formation in the previous cycle.

In Experiment 1, all genotypes tolerated irrigation with ECw of 9.0 dS m^{-1} in vegetative phase (management strategy $A_2B_1C_1$). However, in experiment II, submitted to irrigation with salinized water in the vegetative phase, the genotypes 'BRS Rubi' and 'BRS Topázio' were classified as moderately sensitive, whereas 'BRS Safira' was moderately tolerant when submitted to strategies B-EV and BC-EV, whereas in plants from seeds produced with salt stress in the yield formation phase, in the C-EV strategy, as in the first experiment, it was classified as tolerant (Table 4). These results indicate the presence of wide genetic variability for salt tolerance among the studied genotypes, reducing the tolerance of 'BRS Rubi' and 'BRS Topázio'. Ultimately,

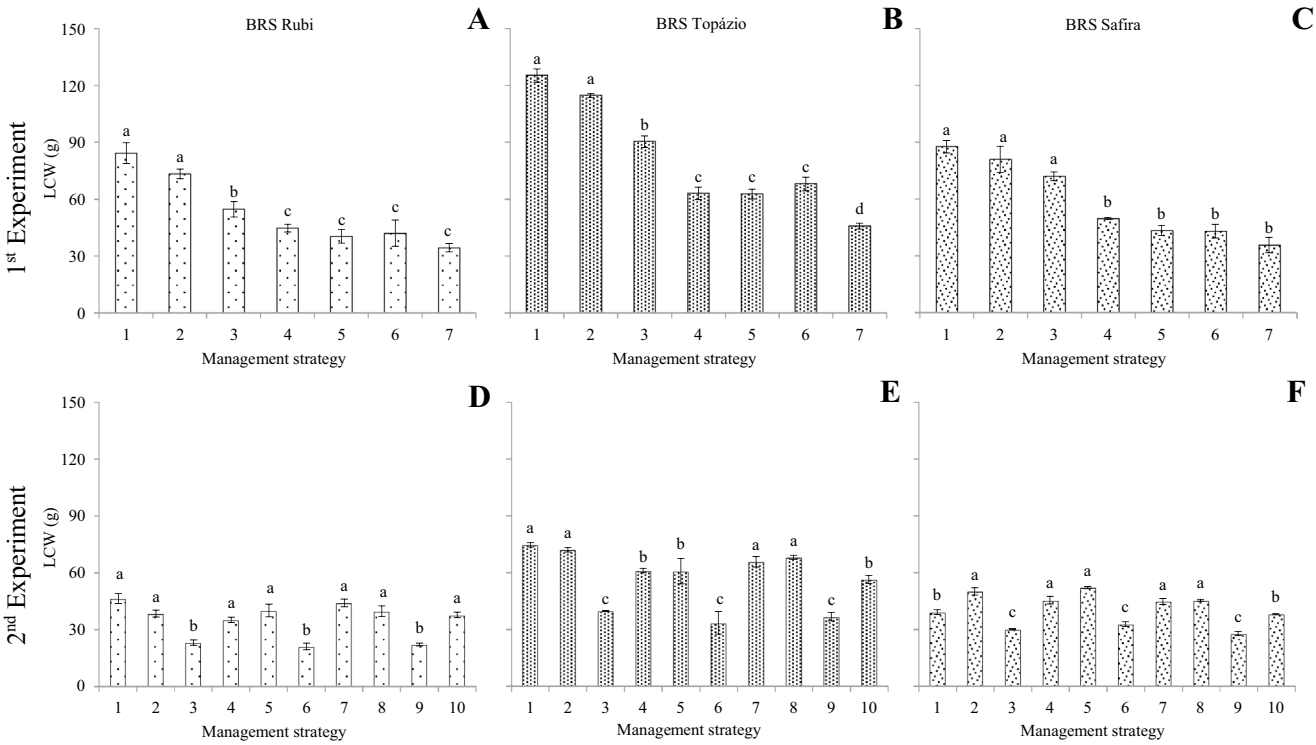

Fig. 3 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for lint cotton weight (LCW) in the first (**a**, **b** and **c**) and second (**d**, **e** and **f**) experiments, respectively. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

salinity in the flowering and yield formation period lowers yield and lowers vegetative salt tolerance in the following year.

Table 5 presents the classification of genotypes based on the criterion of relative reduction of lint cotton weight, comparing the yields obtained at the salinity level of 9.0 dS m^{-1} in the flowering, yield formation and flowering/yield formation phases, in comparison to the production achieved in plants irrigated with low-salinity water (0.8 dS m^{-1}), in both experiments. Based on this evaluation criterion, it was found that 'BRS Rubi' and 'BRS Topázio' were moderately tolerant to irrigation with 9.0 dS m^{-1} water in the flowering phase while 'BRS Safira' was tolerant. However, the salt tolerance of 'BRS Safira' improved in the following. In Experiment I, all genotypes studied were considered moderately sensitive to high level of water salinity, but in Experiment II, they showed higher levels of tolerance during the yield formation stage. When salt stress was applied in succession during the flowering and yield formation phases, 'BRS Rubi' and 'BRS Safira' were moderately sensitive and 'BRS Topázio' was sensitive. However, in the second year, the salt tolerance of the genotypes improved to tolerant (BRS Rubi and Safira) and moderately tolerant (BRS Topázio).

Fiber percentages (%Fiber) were similar between the colored cotton genotypes, in both experiments (Fig. 4). In the first cycle, saline water irrigation during the flowering and yield formation stages affected only the genotype 'BRS Rubi', causing reductions of 14.35%, 8.32%, 10.34% and

Table 4 Classification of cotton genotypes for tolerance to salinity in the vegetative phase by the criterion of relative reduction of lint cotton weight, in joint analysis, of Experiments I and II

Genotypes	Relative reduction (%)	Reduction range	Classification	Relative reduction (%)	Reduction range	Classification
	$A_2B_1C_1$ (Experiment I)			B-VS (Experiment II)		
BRS Rubi	13.04	< 20	T	50.72	40–60	MS
BRS Topázio	8.51	< 20	T	47.08	40–60	MS
BRS Safira	7.60	< 20	T	23.27	20–40	MT
	C-VS (Experiment II)			BC-VS (Experiment II)		
BRS Rubi	55.08	40–60	MS	52.89	40–60	MS
BRS Topázio	55.60	40–60	MS	51.12	40–60	MS
BRS Safira	16.37	< 20	T	29.31	20–40	MT

Experiment I: Index 1 (A_1, B_1, C_1): irrigated with low-salinity water (0.8 dS m^{-1}); Index 2 (A_2, B_2, C_2): irrigated with high-salinity (9 dS m^{-1})

Experiment II: B—seeds from plants irrigated in the previous cycle with salinized water at flowering, C—seeds from plants irrigated with salinized water at yield formation and BC—seeds produced in the first experiment under high salinity at flowering and yield formation; VS: high-salinity in the vegetative stage

T = tolerant; MT = moderately tolerant and MS = moderately sensitive

Table 5 Classification of cotton genotypes for salinity tolerance in the flowering, yield formation and flowering/yield formation phases by the criterion of relative reduction of lint cotton weight, in joint analysis, of Experiments I and II

Genotypes	Relative reduction (%)	Reduction range	Classification	Relative reduction (%)	reduction range	Classification
	Experiment I			Experiment II		
Flowering						
BRS Rubi	35.17	20–40	MT	24.63	20–40	MT
BRS Topázio	27.92	20–40	MT	18.38	< 20	T
BRS Safira	17.87	< 20	T	–	< 20	T
Yield formation						
BRS Rubi	47.03	40–60	MS	5.02	< 20	T
BRS Topázio	49.73	40–60	MS	12.10	< 20	T
BRS Safira	43.34	40–60	MS	–	< 20	T
Flowering/Yield formation						
BRS Rubi	59.28	40–60	MS	18.84	< 20	T
BRS Topázio	63.56	> 60	S	24.66	20–40	MT
BRS Safira	59.31	40–60	MS	2.58	< 20	T

T tolerant; *MT* moderately tolerant; *MS* moderately sensitive, *S* sensitive

13.15% under the strategies $A_1B_2C_1$, $A_1B_1C_2$, $A_2B_2C_1$ and $A_1B_2C_2$, respectively, compared with plants under low-salinity water (Fig. 4a, b, c). In the second experiment, fiber percentage data showed a similar trend to that of lint cotton weight, i.e., salt stress applied during flowering and yield formation stages did not affect %Fiber, which was equal to 39.29%, 36.75% and 38.17% under the strategies T4—B-FLS, T7—C-YFS and T10—BC-FFS. In the strategies T4 and T10, %Fiber increased by 7.57% and 3.23% without differing from the values obtained with low-salinity water, corresponding to the strategies B-S0, C-S0, and BC-S0 (Fig. 4d, e, f).

Among the genotypes evaluated in the first experiment, conducted without application of salt stress ($A_1B_1C_1$), greater fiber length was found in 'BRS Topázio' (29.67 mm), differing from 'BRS Rubi' and 'BRS Safira', whose UHM values were 23.08 and 24.09 mm, respectively (Fig. 5a, b, c). No significant variation in UHM was found in 'BRS Rubi' between the salinity management strategies (Fig. 5a). The genotypes 'BRS Topázio' and 'BRS Safira', when subjected to salt stress during the yield formation stage, had reductions in fiber length compared with plants irrigated with low-salinity water. In the strategies 4—$A_1B_1C_2$, 5—$A_2B_1C_2$, and 7—$A_1B_2C_2$, the reductions in UHM were 5.58%, 6.52%, and 4.81% in 'BRS Rubi' and 12.31%, 4.01% and 6.86% in 'BRS Safira', respectively, compared with the strategy without salinity (Fig. 5b, c).

In Experiment II, the treatments with saline water imposed on the production of seeds in Experiment I did not differ in the new cycle, except for the genotype 'BRS Safira', which had greatest fiber length under saline water application during the flowering and yield formation stages (10—BC-FFS). Greatest fiber length was found in the genotype 'BRS Topázio', with mean value of 30.90 mm, which was 24.16% and 23.12% higher than those observed in 'BRS Rubi' and 'BRS Safira', respectively (Fig. 5d, e, f). Among the genotypes studied, regardless of the management strategies of saline water application in both experiments, 'BRS Topázio' showed greater fiber length uniformity (85.69% in the first experiment and 85.63% in the second experiment), surpassing 'BRS Rubi' and 'BRS Safira' (Fig. 6a, b).

The interaction between salinity management strategies and genotypes had significant effect on short fiber index (SFI) in both years of cultivation (Fig. 7a, b), with variations between the strategies only in the genotype 'BRS Safira'. Highest SFI in this cultivar was found in plants subjected to salt stress during the yield formation stage ($A_1B_1C_2$) in the first experiment (SFI of 12.18%); in

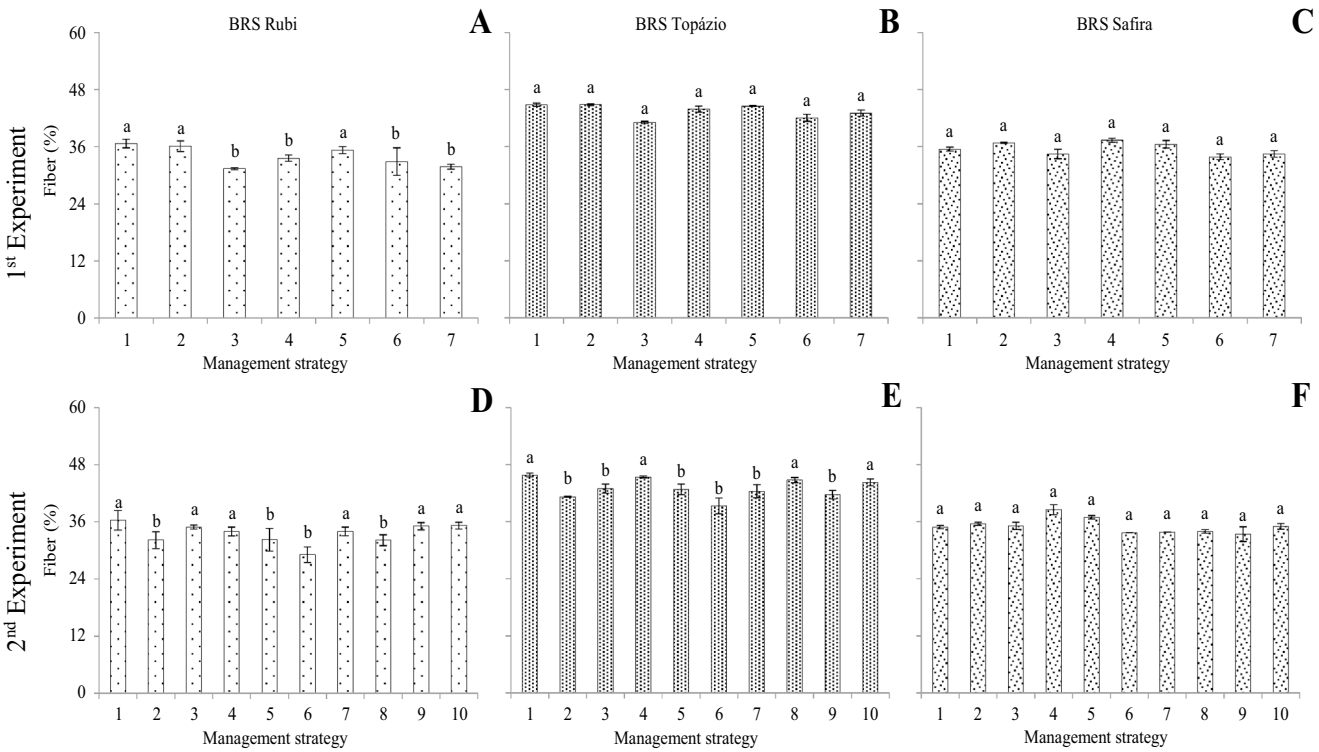

Fig. 4 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for fiber percentage (%Fiber) in the first (**a**, **b** and **c**) and second (**d**, **e** and **f**) experiments, respectively. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

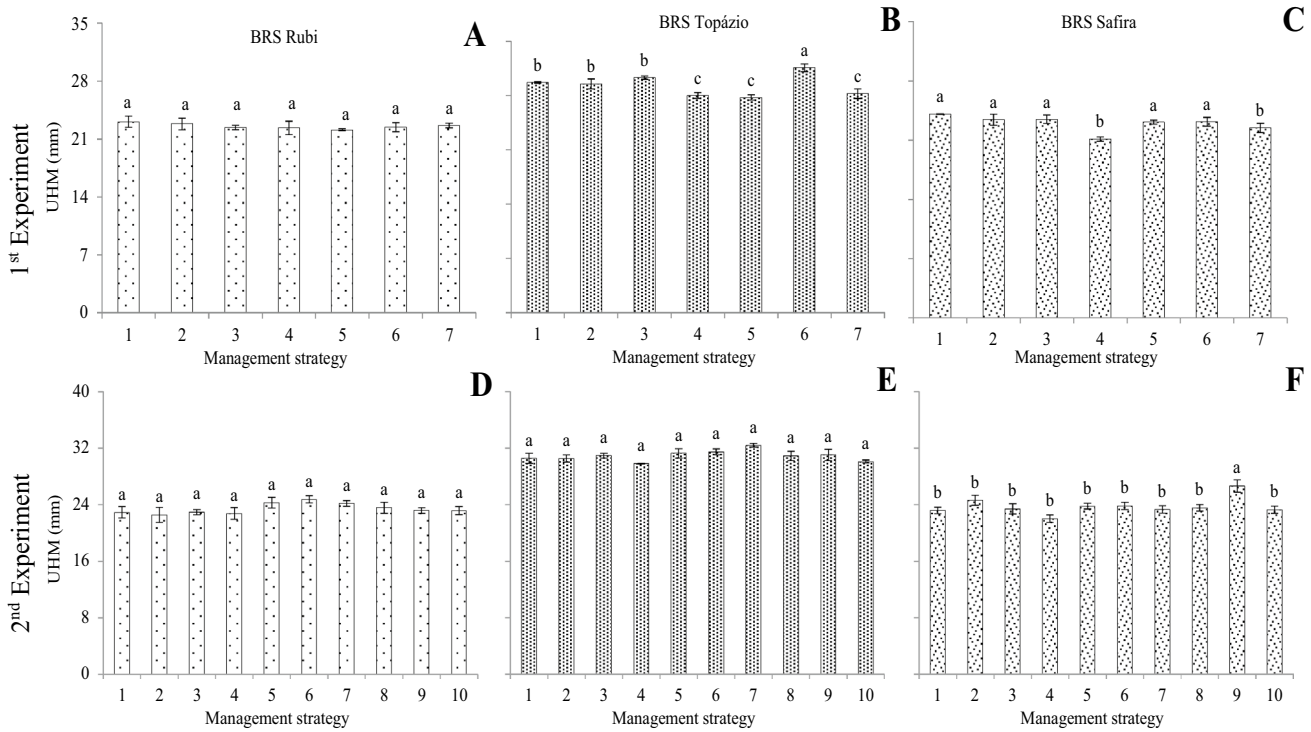

Fig. 5 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for fiber length (UHM) in the first (**a**, **b** and **c**) and second (**d**, **e** and **f**) experiments, respectively. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

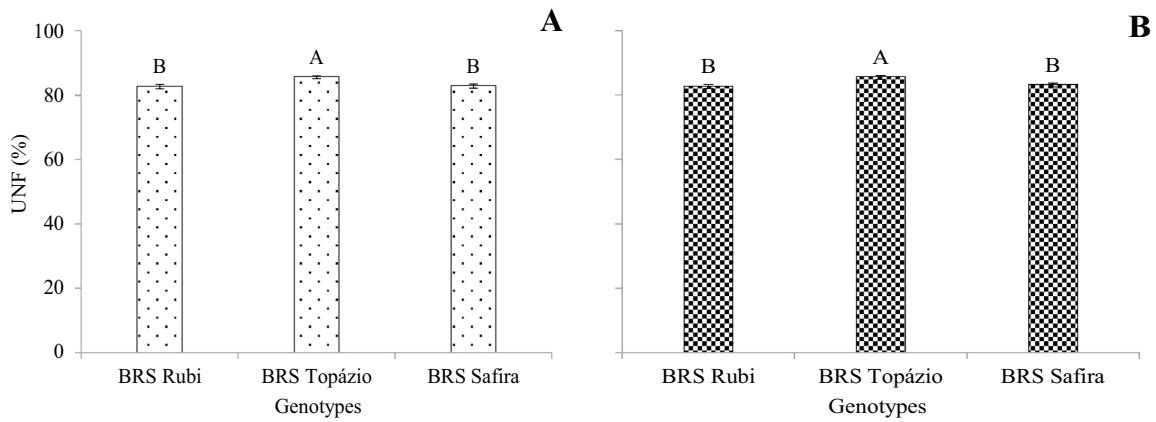

Fig. 6 Test of means for fiber length uniformity (UNF) between the cotton genotypes in the first (**a**) and second (**b**) experiments, respectively. Genotypes with the same letter do not differ by Tukey test, $p < 0.05$

Experiment II, the SFI of 'BRS Safira' plants under salt stress, also during the yield formation stage, decreased to 7.95%.

In relation to oil content, the simple effect analysis of salinity management strategies for each cotton genotype studied in the first year of cultivation revealed higher values in 'BRS Rubi' (25.52%), differing from 'BRS Topázio' and 'BRS Safira', which showed reductions in oil content of 23.94% and 23.81%, respectively (Fig. 8a, b, c). However, when the genotypes were irrigated with high salinity water (9 dS m^{-1}) during the flowering and yield formation stages, under the strategies $A_1B_1C_2$,

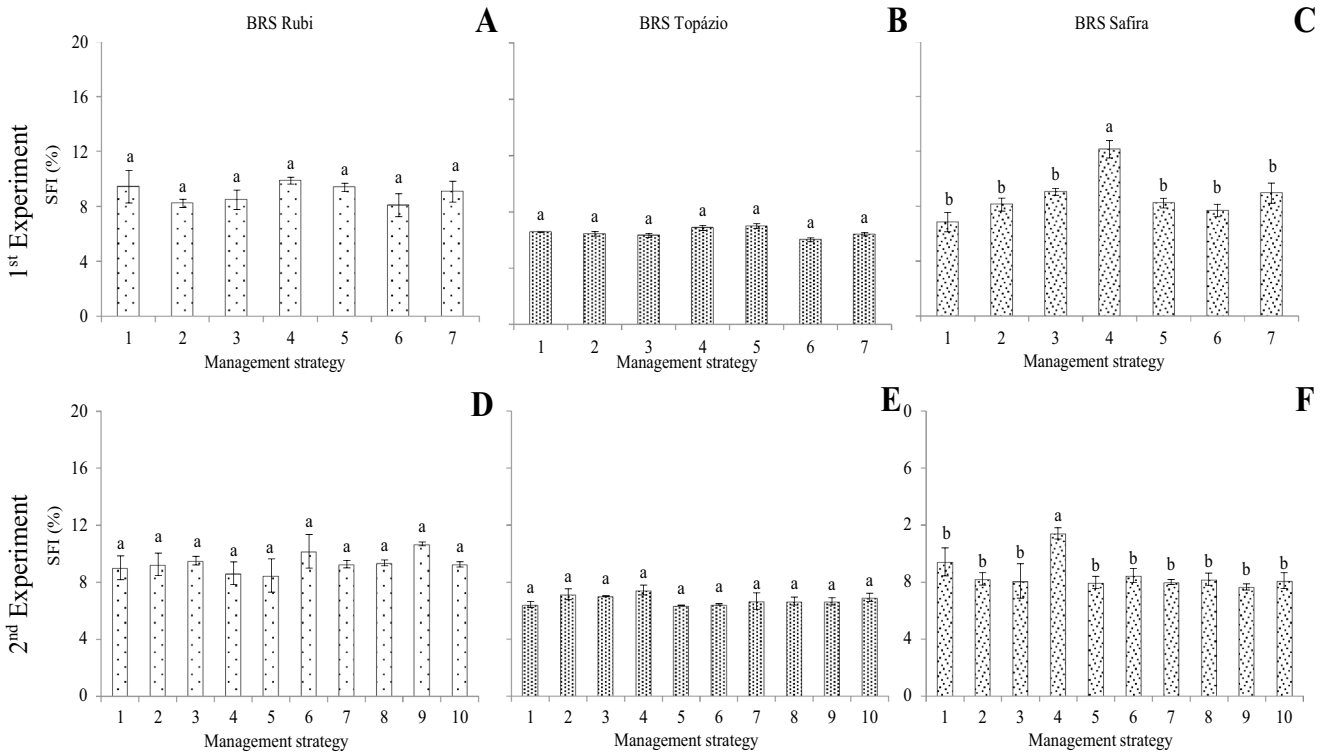

Fig. 7 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for short fiber index (SFI) in the first (**a**, **b** and **c**) and second (**d**, **e** and **f**) experiments, respectively. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

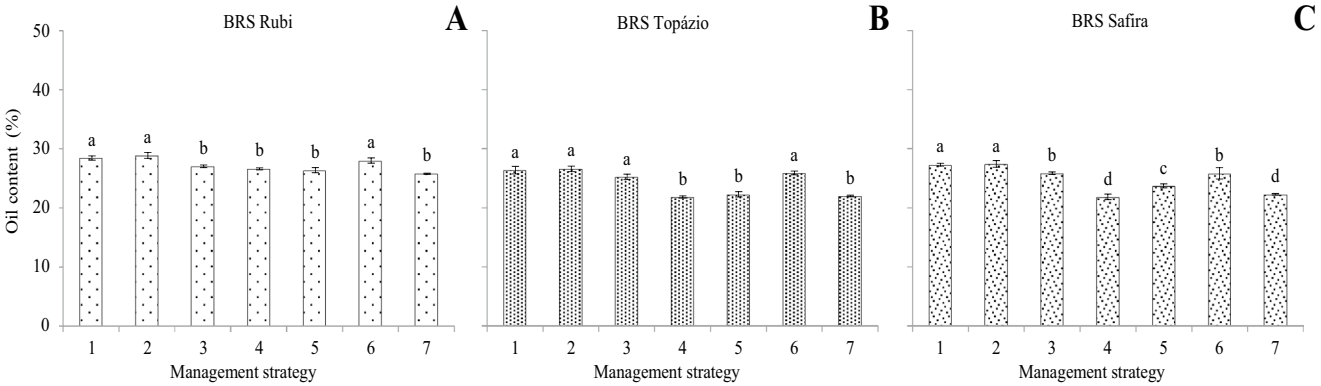

Fig. 8 Test of means in the simple effect analysis of the interaction between genotypes and management strategies for oil content in the first experiment. In each management strategy, bars with the same lowercase letter indicate no significant difference between the means of salinity treatments (Scott–Knott, $p < 0.05$)

$A_2B_1C_2$ and $A_1B_2C_2$, their oil contents decreased by 14.46%, 11.93% and 14.70% respectively, compared with plants under no salinity along the cycle. In all cotton varieties subjected to salt stress only during the vegetative stage, with the management $A_2B_1C_1$, the oil contents were similar to those of plants cultivated in the absence of salt stress (Fig. 8), which had highest oil content per plant (27.54%).

Discussion

The cotton genotypes showed contrasting responses to different salinity management strategies in successive cycles, proving that the survival of plants under adverse conditions will depend largely on the regulation of genes responsive to stress, that is, the activation of epigenetic

mechanisms during the development of the plant in response to environmental stimuli without changes in the sequence of their DNA (Tan 2010). Thus, the fact that the interaction MS x G was not significant for fiber length uniformity and oil content reinforces the idea that the treatments corresponding to saline water irrigation in the second production cycle caused no changes in the genotypes evaluated. The results for seed cotton weight agree with those obtained by Cavalcante et al. (2005), for two cotton cultivars, CNPA-7H and brown-colored BRS-200, under irrigation with saline water along the entire cycle, and observed that the yields of both cultivars were compromised by water salinity higher than 3.1 dS m^{-1}.

Using saline water during flowering and yield formation stage possibly did not interfere with cotton production, because these plants were grown from seeds which had already been exposed to saline conditions in Experiment I. In contrast, when analyzing cotton recovery after plant exposure to different levels of water salinity (2, 10 and 20 dS m^{-1}), in different growth stages, Khorsandi and Anagholi (2009) observed lesions in plants, under conditions of moderate (10 dS m^{-1}) to high salt stress (20 dS m^{-1}) in the vegetative phase, and cotton plants did not recover properly. Therefore, in the management of irrigation, water with low levels of salinity should be used until the complete establishment of the cotton plants.

For the first cultivation, when irrigation with saline water was performed in the flowering phase ($A_1B_2C_1$) and yield formation ($A_1B_2C_2$), at the end of the cycle the weight of cotton lint was reduced compared to plants irrigated with 0, 8 dS m^{-1} water throughout the cycle. These are an aggravating factor, since the water requirement in the flowering and yield formation phases are greater when compared to the vegetative phase; with this more salts are incorporated into the soil, compromising cotton yield (Loka and Oosterhuis 2012). Conversely, plants grown from seeds formed under high-salinity conditions in the stages of flowering and yield formation, in the previous cycle, were better adapted to the salt stress in the new cycle during flowering and fruiting only, and their production levels were similar to those of plants under no salt stress, despite being irrigated with high-salinity water in the stages of flowering (T4—B-FLS) and yield formation (T7—C-YFS). Differently, Min et al. (2016) analyzed white fiber cotton submitted to three salinity levels of irrigation water (0.35, 4.61, and 8.04 dS m^{-1}) and two rates of N application (240 and 360 kg N ha^{-1}) during five production cycles, and found that treatment with 4.61 dS m^{-1} of water increased growth, productivity and N absorption in the first year, but in the third, fourth and fifth productive cycles, growth, yield and N absorption were significantly lower in the treatment with 4.61 dS m^{-1} water than in the treatment with 0.35 dS m^{-1} water. These data are of great importance because, in the three genotypes, there was evidence of cotton adaptation to salt stress and consequences on plant genetics, with effects on a new life cycle. Possibly, when seeds were formed under high-salinity levels, there was expression of genes which remained active in new individuals in the following cycle, resulting in improved tolerance to salt stress.

This study opens research lines involving gene expression, aiming to adequately interpret what has occurred on molecular level. The different results observed in the treatments in which the salinity level, in the second cycle, was applied during the vegetative stage (3, 6 and 9), are one of the challenges to be investigated to find out what has occurred with the genes. Acquired epigenetic characteristics resulting from epimutation formation are defined by genes associated with stable DNA methylation or histone methylation marks that control transgenerational hereditary phenotypic characteristics without altering genomic DNA sequences; therefore, epigenetics has important implications for reevaluating the theory of evolution, including various chemical modifications of chromatin, which promote or repress specific gene expression in response to abiotic stresses without altering the underlying genetic code (Jones 2012; Richards 2017). Given this, epigenetic variation within a population increases plant production compared to epigenetically uniform populations (Latzel 2013; Song 2017). Chen and Zhou (2013) identified differentially methylated genes between wild and cultivated cotton that potentially contributed to the domestication characteristics, including flowering period and seed dormancy. Osabe et al. (2014), studying DNA methylation in cotton genotypes, observed that cotton is susceptible to epigenetic regulations responsible for phenotypic diversity and, consequently, for enhancement in agronomic performance.

These differences due to salinity management strategies in the second experiment indicate that successive reproduction may result in improved tolerance to salinity in colored cotton genotypes. Reductions in fiber length as a result of the application of salinity may be related to genetic factors and may also be influenced by environmental factors (Meredith 1984). It is important to emphasize that the results of fiber length of cotton plants under salinity are controversial because, while some studies found no differences when plants were subjected to salinity (Rhoades et al. 1988), others have reported increments in UHM, as the concentration of salts in irrigation water increased (Ray et al. 1989). Apparently, a succession of changes in fiber quality is explicitly related to the highest %Fibers, UHM and UNF in 'BRS Topázio', which is justified by the variation in the genetic expression of each genotype. The values of fiber uniformity obtained in this study are of great interest because they were high (above 85%) (Cotton Incorporated 2001). Maintaining the trends already observed for fiber percentage and length,

the lowest percentages of short fiber (6.62%) were found in the genotype 'BRS Topázio', regardless of the salinity management strategies, and these values were classified in the category of low content of short fibers (Fonseca and Santana 2002).

The fiber length uniformity was unaffected by salinity, which is positive because this index impacts the technological quality of the fiber (Pedroza et al. 2006). Ahmed et al. (2007) observed no significant reduction in the oil content of cotton plants cultivated in saline soil with ECse of 11.0 ± 0.55 dS m^{-1}, different from what was found in the present study, which may be related to the differences in management of the cotton genotypes used. Firmino et al. (2007) state that there is a high variation in oil content between colored cotton and white fiber genotypes and found that colored cotton cultivars had higher oil contents than conventional white fiber cultivars, confirming oil content of 24.28% and 23.5% in the BRS Rubi and BRS Safira cultivars, respectively, surpassing the white fiber cotton with 14.5%. The values are above the world average in relation to seed (14.5%) and these genotypes can be used for food and biofuel production purposes (Beltrão and Araújo, 2004). In other words, after the stage of vegetative growth, in which the sensitivity to salt stress is more evident, cotton plants become progressively tolerant throughout the cycle (Lauchli and Epstein 1990; Maas and Grattan 1999).

Conclusions

In the first crop cycle, the 'BRS Rubi', 'BRS Topázio', and 'BRS Safira' genotypes are moderately sensitive to salt stress during the flowering and yield stage. However, in one cycle, the salt tolerance improved in the flowering and yield formation stages. In the second production cycle, colored cotton plants grown from seeds formed under salt stress in the stages of flowering and yield formation had increments in lint cotton weight and fiber quality. Oil content in the cotton genotypes was not affected in the second year by the stress caused through cumulative salt amounts, considering seeds produced in plants irrigated with saline water in the previous cycle.

Acknowledgements To the National Council of for Scientific and Technological Development Council—CNPq, for granting the doctoral scholarship to the first author and Embrapa Cotton, for supplying the seeds of colored cotton.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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