ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 15, Issue, 02, pp.23760-23767, February, 2023
DOI: https://doi.org/10.24941/ijcr.44678.02.2023

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

RESISTANCE MECHANISMS OF TWELVE VARIETIES OF COTTON (*GOSSYPIUM HIRSUTUM* L.) TO WATER DEFICIT INDUCED DURING THE FLOWERING STAGE IN TOGO

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ARTICLE INFO

Article History:
Received 14th November, 2022
Received in revised form
17th December, 2022
Accepted 19th January, 2023
Published online 19th February, 2023

Key words:
Drought, Cotton; Tolerance; Variety; Togo.

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ABSTRACT

Background: One of the limiting factors of cotton production in West Africa, particularly in Togo, is drought. Identifying the resistant varieties and understanding the mechanisms involved in their resistance is a fundamental asset for the breeder. ***Objectives:*** Theaims of this research were to: (i) compare the resistant and susceptible varieties under water deficit conditions on the agro morphological and biochemical levels and (ii) identify the traits responsible for water deficit resistance. ***Methods:*** Twelve varieties of cotton (*Gossypium hirsutum* L.) were studied under two water regimes (normal and deficit during flowering), in 30 L plastic pots, under controlled sowing conditions, following a split-plot design with three replications. The observations were made on agromorphological and biochemical characteristics. ***Results**:* The results showed that water deficit decreased seed cotton production (-23%), aerial biomass (-29%), root biomass (-41%), vegetative branches bolls (-49,56), root volume (-26,34%), and chlorophyll content (-79% to 79%). On the other hand, the water deficit increased the average weight of the bolls (+23%). The resistant varieties to the water deficit were characterized by an increase in the primary root length (variety X148), the chlorophyll concentration (variety STONE 907), the lateral root number, and proline content (variety BRS 286), and a decrease in the MDA content (STONE 907). The susceptible varieties were characterized by high MDA content (variety NTALL88) and a low lateral root number (variety Deltatpine SL Frego). ***Conclusion:*** STONE 907, BRS286, and X148 can be used as progenitors in crossing programs to improve the water deficit resistance of cotton varieties grown in Togo and West African countries.

Citation: ***Kokou Zovodu KOFF, Atalaèsso BOKO BANA, Mawuli AZIA DEKEY, Nambou GNOFAM et al.*** **2023.** "Resistance mechanisms of twelve varieties of cotton (*Gossypium hirsutum L.*) to water deficit induced during the flowering stage in Togo*". International Journal of Current Research*, 15, (02), 23760-23767.

INTRODUCTION

Cotton is grown mainly for its fiber and seeds, and its production represents an essential source of income for African countries, especially Togo, where cotton is the main cash crop (MAEP, 2013). However, the sustainability of cotton production depends on many biotic and abiotic constraints. Among the abiotic constraints, drought occupies an important place by the frequency of its occurrences and the significant losses of cotton production in quality and quantity that it causes (Sultan *et al*., 2008; Gnofam *et al*., 2014; Lacape *et al*., 2015; Gnofam *et al*., 2022). In Togo, studies reveal a decreasing trend in rainfall amounts and an increase in the duration of drought episodes during the rainy season due to climate change (Adewi *et al*., 2010; TCNCC, 2015; Djaman *et al*., 2017).

Several adaptation strategies have been developed to limit the negative impacts of drought on cotton production. Among them, varietal creation occupies a primary place (Lacape *et al*., 2015; Rauf *et al*., 2016; Bokobana, 2017; Khan *et al*., 2018; Gnofam *et al*., 2021). For this purpose, between 2018 and 2019, one hundred and eighteen (118) cotton varieties were studied under normal water supply conditions and induced water deficit for 30 days during the flowering phase in Togo, and the tolerant varieties were identified (Koffi *et al*., 2021). However, the tolerance mechanisms of these varieties still need to be discovered and constitute a challenge for breeders better to exploit these varieties as progenitors in crossing programs. As in many cultivated plants, water deficit induces numerous adaptation mechanisms in cotton. Many authors have shown that the key responses against drought stress are: root development, stomatal closure, photosynthesis, osmolyte production (proline, glycine betaine, sugar), and activation of genes involved in the scavenging of reactive oxygen species (Farooq *et al*., 2009; Loka *et al*., 2011; Comas *et al*., 2013; Uhlla *et al*., 2017; Hasan *et al*., 2018; Mahmood *et al*., 2020). The general objective of this study is to contribute to improving cotton productivity. More specifically, it will aim at (i) comparing the behavior of resistant and susceptible varieties under water deficit conditions on the agromorphological and biochemical levels and (ii) identifying the traits responsible for water deficit resistance of cotton in the Gossypium hirsutum collection available in Togo.

MATERIAL AND METHODS

Study site: The trial was conducted under controlled sowing conditions on the experimental station of « Centre de Recherche Agronomique de la Savane Humide de Kolokopé » (N 7°47'56"; E 1°17'38"). The climate is intermediate between the two main climates of the country (tropical guinean in the South and tropical sudanian in the North).

Plant material: The plant material consisted of 12 genotypes of G. hirsutum, selected based on the results of previous trials (Koffi *et al*., 2021) (Table 1).

Table 1. Lists of varieties selected for the trial

N°	Varieties	Origins	Classes
1	AF 401-13	Togo	Water stress-tolerant with high production potential
2	Deltapine 5690	USA	
3	Nazili	Turkey	
4	STONE 907	USA	
5	AJ 275 B	Togo	Susceptible to water stress with high production potential
6	STAM 190	Togo	
7	BRS 286	Brazil	
8	STAM 129A	Togo	
9	Deltapine SL Frego	USA	Susceptible to water stress with low production potential
10	NTAL 88	Mali	
11	X148	Ivory Coast	Water stress-tolerant with low production potential
12	FK 64	Burkina Faso	

Experimental design: The experimental design used was the split-plot with two factors and three replications. The main factor was the water regime with two variants: normal water supply (ETM) and water supply with a 30-day deficit induced during the flowering phase (STR). The secondary factor was the variety with 12 variants. The experimental unit consisted of one pot. The culture of the cotton plant was carried out under semi-controlled conditions in plastic pots of 30 L (41 cm depth, 28 cm lower diameter, 34 cm upper diameter). The bottom of these pots was perforated to let the water drain after watering. The pots were placed on polypropylene sheets to prevent the roots from sinking into the soil. In each pot, 2 kg of gravel was poured at the bottom, and the remaining volume was filled with 28 kg of soil taken from the 0 to 20 cm horizon on the E3 strip of Kolokopé Experimental Station. This soil was of sandy-clay type (Table 2).

Table 2. Physico-chemical characteristics of the soil used as a substrate for the test

Parameters	Content
Clay	14,18 %
Fine silt	3,8 %
Coarse silt	6,53 %
Fine sand	44,8 %
Coarsesand	27,43 %
Field capacity (ϴfc) pF2,5 (0,3 bar)	23,53 %
Permanent wilting point (ϴwp) pF4,2 (15 bar)	20,52 %
Density	1,09

We induced the water deficit at the flowering stage of the plant (as soon as the first flower appeared) by decreasing the amount of water from 70% of the total available soil water (TAW) (control treatment) to 30% of the TAW (stressed treatment). Allen *et al*. (2006) shows that generally to cotton, when the soil moisture is under 35% of the TAW, cotton is water stressed. We applied the water deficit for 30 days, corresponding to the average duration of drought pockets recorded during the last crop cycles in Togo (Ledi *et al*., 2020). At the end of the water shortage cycle, irrigation was resumed for the control, i.e., at 70% of the TAW, until the boll opening. The TAW was calculated according to the following formula (Baize, 2000; Bokobana *et al*., 2019):

TAW=(ϴfc 2,5 - ϴ wp 4,2) x T fine x E x Da

TAW: Total available soil water in mm.cm-1; ɵ fc2,5: Moisture at field capacity in %, ɵ wp4,2: Moisture at the permanent wilting point in %; Tfine: % in fine particles; E: soil depth in dm; Da: apparent soil density in t.m-3. The irrigation of the plants is done by successive weighing of the pots at a periodicity of 3 days. During each weighing, the volumes of water corresponding to the different treatments are adjusted.

Conduct of the trial: The cotton seed was sowed on September 06, 2021, at a rate of 3 seeds per pot, followed by the thinning of one plant per pot 15 days after sowing (d.a.s.). The fertilizer NPKSB (12-18-20-5-1) was applied at a rate of 200 kg/ha (4.8 g per pot) before sowing, and urea (46% N) was applied at a rate of 50 kg/ha (1.2 g per pot) at 35th d.a.s. Three insecticide treatments were made each week from the 35th d.a.s to the opening of the first bolls (25 treatments in total). The products used were Cypermethrin/Abamectin (72/20 g/l) at a rate of 0.5 l/ha against bollworms and mites (first nine treatments) and bifenthrin/acetamiprid (120/32 g/l) at a rate of 0.25 l/ha against bollworms, feeder, and sucking insect (last 16 treatments).

Measured parameters: Measured parameters were morphological (aerial and root parts), agronomic and biochemical. Morphological and agronomic parameters measurement. Morphologically, the measured parameters were plant height (PH), vegetative branches number (VB), fruiting branches number (FB), vegetative branches bolls number (VBB), fruiting branches bolls number (FBB), mainstem circumference (MSC),primary root length (PRL), lateral root number (LRN) and root volume (RV). At the agronomic level, the measured parameters were the seed cotton production per plant (SCP), the aerial biomass (AB), root biomass (RB), the average weight of the bolls (WB), the number of seeds per boll (NSB), the first flower appearance date (FFAD) and the first boll opening date (FBOD)

Biochemical parameters Measurement

The measure of total Protein and chlorophyll pigments content: Total leaf protein was determined by the Bradford method (Bradford, 1976). Chlorophyll pigments were extracted by solubilization in 80% acetone (Queval *et al*., 2007). Chlorophyll a, chlorophyll b, and total chlorophyll concentrations expressed as µg-mL-1 were determined by the formulas of Arnon (1949):

Total chlorophyll (µg-mL-1) = 20.2 (A645) + 8.02 (A663)
Chlorophyll a (µg-mL-1) = 12.7 (A663) - 2.69 (A645)
Chlorophyll b (µg-mL-1) = 22.9 (A645) - 4.68 (A663)
An: absorbance of the chlorophyll solution at wavelength n
The measure of malondialdehyde (MDA) content

The extraction and determination of MDA were carried out according to the method of Heath and Paker (1968). Thus, 250 mg of fresh plant material was taken and ground. The grind was homogenized in 5 ml of 5% (w/v) trichloroacetic acid containing 1.25% glycerol. The homogenate was centrifuged at 12,000 rpm for 10 minutes and filtered through Whatman #1 paper. The supernatant was collected in test tubes. To 2 ml of supernatant was added 2 ml of 0.67% thiobarbituric acid, prepared in distilled water. The mixture was homogenized by vortexing and then incubated in a water bath at 100°C for 30 min.

After cooling in melting ice, the mixture was centrifuged under the same conditions for one minute. The absorbance was measured at 532 nm and then at 600 nm. The optical density was then corrected by subtracting the non-specific absorbance at 600 nm. The amount of MDA was calculated using a molar extinction coefficient of 155mM-1 .cm-1, according to the Beer-Lambert law:

$$\mathrm{MDA} = \frac{\mathrm{Absorbance}}{\epsilon \times \mathrm{L}}$$

MDA: Malondialdehyde concentration (mg. g-1 MF); Є: Molar extinction coefficient; L: Cell width (1cm); Leaf proline determination

Measure of leaf proline content: The determination of proline was performed according to the method of Bogdanov *et al*. (1999), modified and adapted to our plant material. 0.5 ml of crude leaf extract (25 mg/ml water) or standard proline solution (32 µg/ml) was taken, and 1 ml of formic acid (100%) and 1 ml of ninhydrin (3%) were added. After vigorous shaking for 15 minutes at room temperature, the mixture in the test tube was boiled for 15 minutes. Then 2.5 ml of 50% 2-propanol was added to the mixture and incubated in a water bath at 70 °C for 10 minutes. After cooling the mixture at room temperature for 45 minutes, the absorbance was read at 510 nm with a spectrophotometer (typeUviline Connect series 940) against a control sample made with distilled water. The proline content of the leaves, estimated in µg.mg-1 of protein, was determined by the following formula:

$$\mathrm{PRO} = \frac{\left(\frac{Ae}{As}\right)\left(\frac{Ms}{Mf}\right)}{TP}$$

PRO: proline content (mg.g-1 of Mf); Ae: Absorbance of leaf extract; As: Absorbance of standard proline solution; Ms: Proline mass of standard solution (µg); Mf: Fresh leaf mass (g); TP: Protein quantity (mg.g.-1 fresh material).

Data processing: Microsoft Excel 2016 spreadsheet was used for data entry and processing. Analyses of variance (ANOVA), discrimination of means via the LSD test at the 5% threshold, and construction of graphs were carried out with the agricolae and ggplot2 packages of R 4.1.3 software. To study the behavior of the resistant varieties concerning the susceptible ones and to balance the information provided by each group of variables, a multiple factor analysis (MFA) was performed with the FactoMiner package of R 4.1.3. Twenty variables were used in this analysis. These variables were grouped into three: agronomic (5), morphological (9), and biochemical (6) variables. All these variables were considered active variables. For a better quality of the representation, only the variables whose sum of the cosines squared in a plane is higher than 0.5 were projected.

RESULTS

Effect of water deficit and varieties on the morphological parameters of the cotton plant: The variance analysis showed that the water regime's effect was significant on the parameters VBB and RV and not on the parameters VB, FB, FBB, PH, MSC, MRL, and SRN (Table 3). The variety effect was significant on VB, FB, FBB, PH, RD, and MRL and not significant on the remaining parameters. The interaction was significant only for the parameters MRL and LRN. Discrimination of means showed that water deficit reduced VBB (-49.56%) and RV (-26.34%) compared to ETM (Table 4). On the varietal level, we noted that the water deficit decreased the VBB for all varieties (-77% to -14%) except for Nazili where we observed an increase of 29%. The results also showed an increase in root volume in the varieties AJ275B (+56%) and BRS 286 (+15%), and a decrease in root volume in the other varieties (-60% to -10%). Discrimination of means showed that under the ETM regime, the number of lateral roots of Deltapine 5690 was the same as those of FK64, AF 401-13, STONE 907, X148, and STAM 190, but higher than the remaining varieties. Under deficit conditions, the value of the lateral root number of the variety BRS 286 was identical to those of the varieties AJ 275B and STONE 907, but higher than those of the remaining varieties. These results also showed that the deficit significantly increased the number of lateral roots in BRS 286 (89%) and reduced the number in AF 401-13 (-44%) and Deltapine 5690 (-44%). The results showed that under the ETM regime, the primary root length of variety FK64 was identical to those of varieties AJ275B, Deltapine SL Frego, BRS 286, AF 401-13, and X148, but, higher than the primary root length of the other remaining varieties. Under deficit conditions, the primary root length of variety X148 was higher than those of the other remaining varieties except for Nazili and BRS 286, which were identical to it. The results also showed that the water deficit significantly decreased the primary root length of varieties Deltapine SL Frego and FK 64 and increased the primary root length of varieties X148, Nazili, and NTALL88 (>15%). Root volume increased significantly in varieties AJ 275B and BRS 286 (> 15%) under water deficit conditions.

Effect of water deficit and varieties on agronomic parameters of cotton: The results of the analysis of variance showed that the effect of the water regime was significant on seed cotton production per plant (SCY), Average weight of the bolls (WB), aerial biomass (AB); root biomass (RB), and not significant on lateral roots number (LRN) and first boll opening date (FBOD) (Table 5). The variety effect was significant on LRN and FBOD parameters and not significant on the remaining parameters, and the interaction was non-significant for all parameters. The discrimination of the means showed that the water deficit decreased SCP (-23%), AB (-29%), and RB (-41%), and increased the WB compared to the ETM on the other hand (table 6). Results showed that the water deficit decreased the SCP in all varieties (-39% to -11%) except BRS 286, for which we noted an increase of +3%. The deficit caused a reduction in all varieties for the variables AB and RB. However, this reduction was small to nil in BRS 286. We noted that the water deficit increased the average boll weight of all varieties except Nazili, where a reduction of -15% in boll weight was observed.

Effect of water deficit and varieties on biochemical parameters of cotton: The results of the analysis of variance showed that the effect of the water regime was significant on the chlorophyll content (ChlT, Chla, and Chlb) and not significant on the total protein content (TP), proline content (PRO), and malondialdehyde content (MDA) (Table 7). The variety effect was significant on all parameters except PRO, and the interaction was non-significant for all parameters. Discrimination of the means showed that the water deficit decreased the chlorophyll contents ChlT (-76%), Chla (-79%), and (-70%) compared to the ETM regime (Table 8). The results showed that the water deficit reduced the chlorophyll content in all varieties, but the reduction was smaller with STONE 907 than with the others.

Grouping of varieties according to their similarity

Choice of dimensions to be considered: Following the principle of KAISER (1960), the first two dimensions (Dim1 and Dim2) of the multiple factorial analysis (MFA) are those whose eigenvalues are greater than 1. They summarize about 54.81% of the observations. However, to extract more information spread over the other dimensions, Dimensions 3 and 4 were also considered in interpreting the data; they summarize 25.89% of the initial information. The first four dimensions sum up 80.7% of the initial information.

Correlation between groups of variables and the main dimensions of the MFA: Three groups of variables were studied. These were agronomic, morphological, and biochemical variables. The results showed that the group of agronomic variables was correlated with dimension 1 (ctr= 43.21%; r= 0.95) and dimension 2 (ctr= 29.72%; r= 0.78). The group of morphological variables was correlated with the dimensions Dim1 (ctr= 21.46%; r=0.90), Dim2 (ctr= 57.41%; r=0.90), Dim3 (ctr= 35.00%; r=0.66) and Dim4 (ctr= 57.41%; r=0.89). The biochemical variables' group was correlated with the dimensions Dim1(ctr= 35.33%; r=0.87) and Dim3 (ctr= 55.03%; r=0.82).

Table 3. Results of analysis of variance of morphological parameters of the aerial and root parts of the cotton plant

Source of variation	DF	Aerial part						Root part		
		VB	FB	VBB	FBB	PH	MSC	PRL	LRN	RV
REG	1	0.031^{ns}	2.92^{ns}	262.587^{**}	63.281^{ns}	2890.53^{ns}	0.272^{ns}	336.22^{ns}	52.14^{ns}	2636.47^{*}
VAR	11	2.83^{*}	10.29^{***}	10.42^{ns}	21.943^{***}	355.49^{***}	0.486^{***}	143.01^{*}	113.44^{ns}	175.12^{ns}
REG x VAR	11	0.895^{ns}	2.102^{ns}	8.738^{ns}	6.539^{ns}	97.79^{ns}	0.106^{ns}	153.08^{*}	161.77^{**}	223.18^{ns}
CVa		43.41	25.09	23.8	33.39	17.35	5.11	82.51	39.06	23.84
CVb		28.1	14.4	62.52	27.39	7.79	7.69	20.83	27.91	35.24

REG: Water regime; **VAR**: Variety; **DF**: Degree of freedom; **VB**: Number of vegetative branches; **FB**: Number of fruiting branches, **VBB**: Vegetative branches bolls; **FBB**: Fruiting branches bolls; **PH**: Plant Height; **MSC**: Main stem circumference; **PRL**: Primary root length; **LRN**: lateral roots number; **RV**: Root volume; **CVa**: Coefficient of variation of the first error; **CVb**: Coefficient of variation of the second error; *******: Very highly significant at the p<0.001 thresholds; ******: Highly significant at the p<0.01 threshold; *****: Significant at threshold p<0.05; **ns**: not significant.

Table 4. Average morphological parameters of the twelve varieties subjected to water deficit

Varieties	**VBB**			**RV (liter)**			**LRN**			**PRL (cm)**		
	ETM	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**
AF 401-13	8,00	2,67	-67	22,00	13,33	-39	36.00 abc	20.00 de	-44	43.00 abcde	38.00 bcdef	-12
AJ 275 B	8,00	3,67	-54	24,00	37,33	56	23.00 cde	31.00 abcde	35	46.60 ab	35.17 bcdef	-25
BRS 286	4,67	4,00	-14	34,33	39,33	15	22.00 cde	41.67 ab	89	44.00 abcde	44.74 abcd	2
Deltapine 5690	5,33	1,67	-69	43,67	17,33	-60	42.34 a	23.67 cde	-44	36.67 bcdef	28.00 f	-24
Deltapine SL Frego	5,50	3,00	-45	35,67	20,00	-44	20.34 de	21.00 de	3	46.34 abc	27.40 f	-41
FK 64	3,67	2,67	-27	34,00	30,67	-10	36.00 abc	23.34 cde	-35	55.34 a	33.84 bcdef	-39
Nazili	2,33	3,00	29	39,33	23,33	-41	20.67 de	23.67 cde	15	37.67 bcdef	45.37 abcd	20
NTAL 88	8,33	3,67	-56	43,33	26,33	-39	18.34 e	24.67 cde	35	33.00 cdef	39.10 bcdef	18
STAM 129A	8,67	2,00	-77	44,33	32,00	-28	29.00 bcde	28.34 cde	-2	34.34 bcdef	34.07 bcdef	-1
STAM 190	7,33	1,33	-82	48,33	27,33	-43	30.00 abcde	26.00 cde	-13	37.67 bcdef	32.00 def	-15
Stone 907	10,33	4,33	-58	44,00	34,33	-22	35.34 abc	32.34 abcd	-8	39.00 bcdef	31.10 ef	-20
X148	7,67	2,00	-74	47,00	19,00	-60	31.00 abcde	25.00 cde	-19	42.34 abcde	53.34 a	26
Mean	**6,65**	**2,83**	**-49,56**	**38,33**	**26,69**	**-26,34**	**41,33**	**36,84**	**0,78**	**28,67**	**26,72**	**-9,08**

Values in the same column with different letters are statistically different at the 5% threshold; ETM: Normal water regime; STR: 30-day water deficit induced during the flowering phase; VBB: Vegetative branches bolls; PRL: Primary root length; LRN: Lateral roots number; RV: Root volume; RD: Relative difference.

Table 5. Results of analysis of variance of agronomic parameters of cotton

Sources of variation	DF	SCP	WB	NSB	AB	RB	FBOD
REG	1	2631.75^{*}	12.27^{*}	1.681^{ns}	6259.6^{*}	504.44^{*}	156.06^{ns}
VAR	11	54.97^{ns}	1.007^{ns}	60.19^{**}	94.60^{ns}	11.78^{ns}	269.91^{*}
REG x VAR	11	129.25^{ns}	0.585^{ns}	34.44^{ns}	104.40^{ns}	11.77^{ns}	153.78^{ns}
CVa		18.53	17.43	8.73	16.96	44.61	12.36
CVb		24.09	19.79	15.25	18.37	37.59	13.39

REG: Water regime; VAR: Variety; DF: Degree of freedom; SCP: seed cotton production per plant; WB: Average weight of the bolls; AB: the aerial biomass; RB: root biomass; NBS: Number of seeds per boll; FBOD: the first boll opening date; CVa: Coefficient of variation of the first error; CVb: Coefficient of variation of the second error;**: Significant at the threshold p<0.01; *: Significant at the p<0.05 threshold; ns: not significant.

Table 6. Average agronomic parameters of the twelve varieties subjected to water deficit

Variety	**SCP (g)**			**BW (g)**			**AB (g)**			**RB (g)**		
	ETM	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**	**ETM**	**STR**	**RD (%)**
AF 401-13	58,00	35,60	-39	3,39	4,64 a	37	69,10	41,53	-40	7,75	6,70	-14
AJ 275 B	59,63	39,77	-33	3,50	4,16 ab	19	67,47	46,43	-31	10,90	6,87	-37
BRS 286	48,05	49,53	3	3,89	4,75 a	22	52,97	49,00	-7	9,43	9,40	0
Deltapine 5690	52,10	41,10	-21	3,34	4,62 a	38	68,30	37,60	-45	13,57	6,33	-53
Deltapine SL Frego	51,17	40,03	-22	3,44	4,09 ab	19	52,07	47,97	-8	10,37	4,23	-59
FK 64	44,83	38,90	-13	3,98	4,61 a	16	62,30	48,37	-22	11,53	7,07	-39
Nazili	60,03	40,63	-32	3,47	2,95 b	-15	51,30	40,00	-22	12,80	6,07	-53
NTAL 88	56,47	35,43	-37	3,34	3,80 ab	14	71,43	46,77	-35	12,20	6,67	-45
STAM 129A	50,77	43,17	-15	3,89	5,31 a	36	67,60	44,73	-34	14,13	10,40	-26
STAM 190	52,10	46,13	-11	4,28	5,00 a	17	71,50	48,40	-32	14,77	6,60	-55
Stone 907	57,17	48,67	-15	3,11	4,87 a	57	65,87	43,87	-33	16,47	6,87	-58
X148	54,67	33,27	-39	3,51	4,23 ab	20	66,27	43,83	-34	11,93	6,40	-46
Mean	**53,75**	**41,02**	**-23**	**3,60**	**4,42**	**23**	**63,85**	**44,88**	**-29**	**12,15**	**6,97**	**-41**

Values in the same column with the same letter or without a letter are statistically identical at the 5% threshold; SCP: seed cotton production per plant; WB: Average weight of the bolls; AB: the aerial biomass; RB: root biomass; ETM: Normal water regime; STR: 30-day water deficit induced during the flowering phase; RD: Relative difference.

Table 7. Result of analysis of variance of biochemicalparameters of cotton

Sources of variation	**DL**	**ChlT**	**Chla**	**Chlb**	**TP**	**PRO**	**MDA**
REG	1	6.545^{**}	3.136^{**}	0.621^{*}	2.309^{ns}	251551^{ns}	4.368^{ns}
VAR	11	0.089^{*}	0.041^{*}	0.010^{*}	0.492^{*}	37118^{ns}	0.541^{*}
REG x VAR	11	0.043^{ns}	1.264^{ns}	0.004^{ns}	0.214^{ns}	36156^{ns}	0.554^{ns}
CVa		48,20	46.09	56.27	11.7	22.09	51.02
CVb		38,26	40.17	35.84	8.94	27.41	16.72

REG: Water regime; VAR: Variety; DF: Degree of freedom; NBL: Degree of freedom; ChlT: Total chlorophyll content, Chla: Chlorophyll a content; Chlb: Chlorophyll b content, PRO: Proline content; TP: Total protein content; MDA: Malondialdehyde content; CVa: Coefficient of variation of the first error; CVb: Coefficient of variation of the second error; **: Highly significant at the threshold p<0.01; *: Significant at threshold p<0.05; ns: not significant.

Table 8. Average discriminant biochemical parameters of the twelve varieties studied according to water regimes

Variety	ChlT			Chla			Chlb			TP			PRO			MDA		
	ETM	STR	RD (%)	ETM	STR	RD (%)	ETM	STR	RD (%)	ETM	STR	RD (%)	ETM	STR	RD (%)	ETM	STR	RD (%)
AF 401-13	0,69	0,11	-84	0,44	0,06	-87	0,26	0,05	-79	6,40	6,16	-4	670,46	517,94	-23	3,55	2,81	-21
AJ 275 B	0,56	0,17	-70	0,37	0,10	-72	0,19	0,06	-67	5,46	6,09	12	632,58	469,20	-26	4,22	3,02	-29
BRS 286	0,96	0,18	-82	0,65	0,11	-84	0,30	0,07	-76	4,73	5,66	20	647,37	758,30	17	2,43	3,34	37
Deltapine 5690	0,79	0,25	-68	0,52	0,15	-71	0,27	0,10	-62	6,01	5,81	-3	578,49	610,79	6	3,78	2,88	-24
Deltapine SL Frego	1,02	0,18	-83	0,68	0,11	-84	0,34	0,07	-81	5,23	5,54	6	626,62	571,52	-9	3,47	2,95	-15
FK 64	0,85	0,15	-82	0,58	0,09	-84	0,28	0,06	-80	5,35	6,04	13	977,18	510,24	-48	4,06	2,74	-32
Nazili	0,92	0,12	-87	0,62	0,06	-90	0,30	0,06	-82	5,26	5,50	5	654,21	559,23	-15	2,78	2,54	-9
NTAL 88	0,57	0,12	-78	0,37	0,07	-82	0,20	0,06	-70	5,61	6,00	7	726,10	546,68	-25	3,64	3,77	4
STAM 129A	0,53	0,20	-61	0,34	0,12	-64	0,19	0,08	-56	5,47	5,57	2	673,31	541,15	-20	3,44	2,93	-15
STAM 190	0,74	0,16	-79	0,52	0,09	-82	0,22	0,07	-71	5,43	6,18	14	782,36	484,75	-38	3,13	3,02	-4
Stone 907	1,12	0,49	-56	0,75	0,34	-55	0,37	0,18	-52	5,38	5,97	11	444,21	456,96	3	3,21	2,56	-20
X148	0,63	0,11	-83	0,43	0,05	-88	0,20	0,06	-71	5,74	5,82	1	518,67	486,22	-6	3,56	2,82	-21
Mean	**0,78**	**0,19**	**-76**	**0,52**	**0,11**	**-79**	**0,26**	**0,08**	**-70**	**5,50**	**5,86**	**7**	**660,96**	**542,75**	**-15**	**3,44**	**2,95**	**-12**

ChlT: Total chlorophyll content, Chla: Chlorophyll a content; Chlb: Chlorophyll b content; PRO: Proline content; TP: Total protein content; MDA: Malondialdehyde content; ETM: Normal water regime; STR: 30-day water deficit induced during the flowering phase; RD: Relative difference.

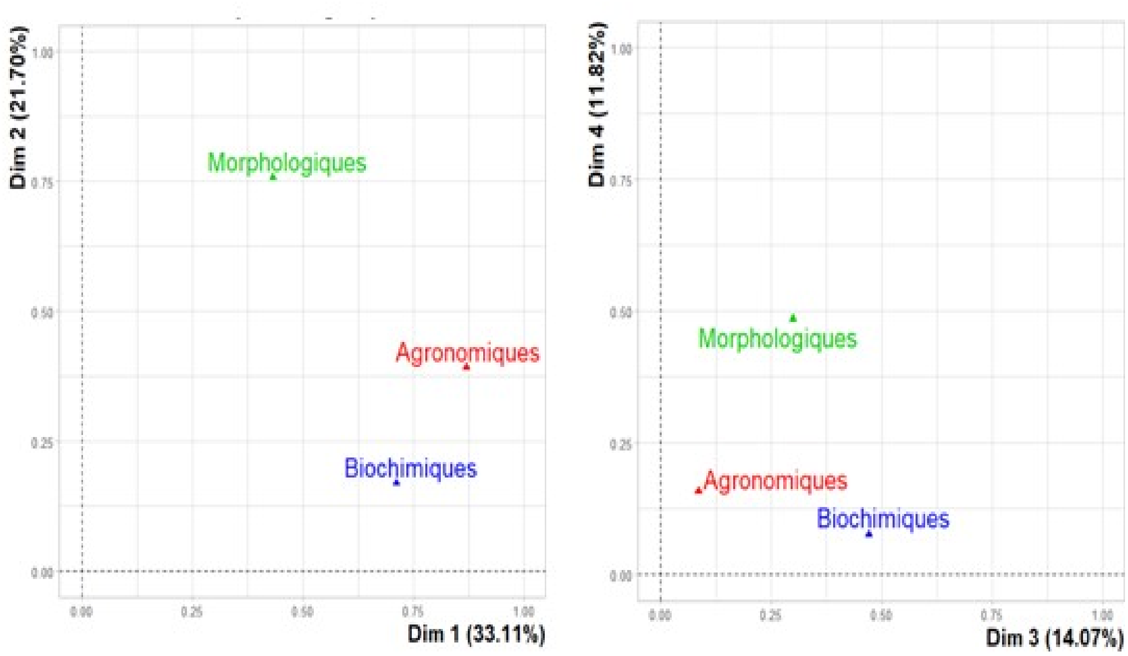

Figure 1. Representation of the groups in the Dim1-Dim2 (left) and Dim3-Dim4 (right) planes

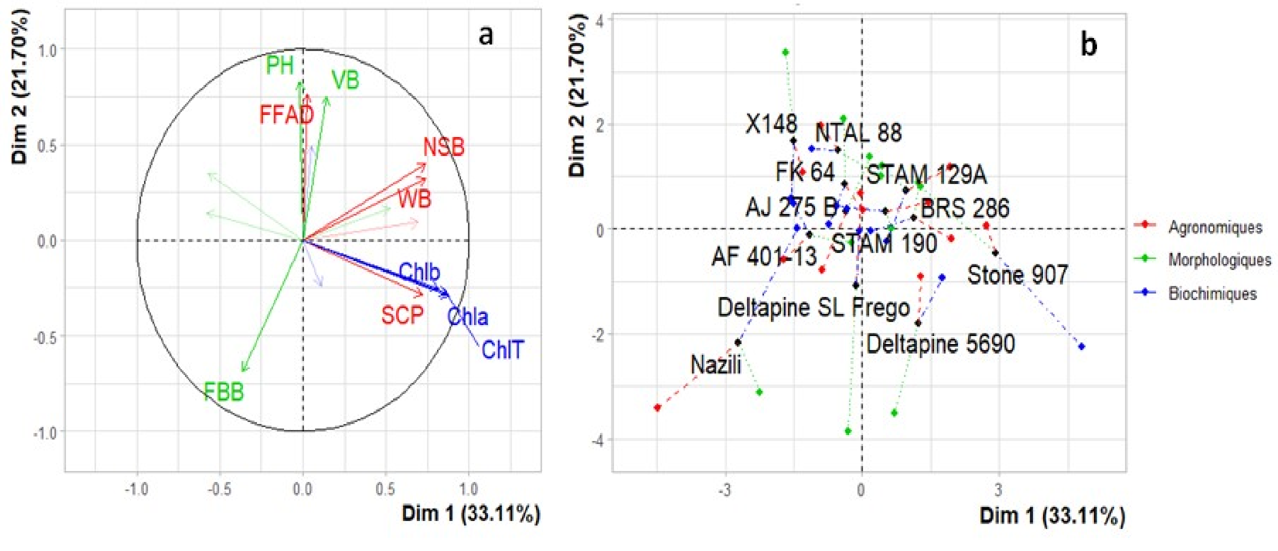

Figure 2. Behavior of variables and individuals in the Dim1-Dim2 plane a- Variables correlations circle; b- Projection of individuals in the Dim1-Dim2 plane.SCP: seed cotton production per plant; WB: Average weight of the bolls; NSB: Number of seeds per boll; FFAD: the first flower appearance date; VB: Number of vegetative branches; FB: Number of fruiting branches; FBB: Fruiting branches bolls; PH: Plant Height; ChlT: Total chlorophyll content; Chla: Chlorophyll a content; Chlb: Chlorophyll b content

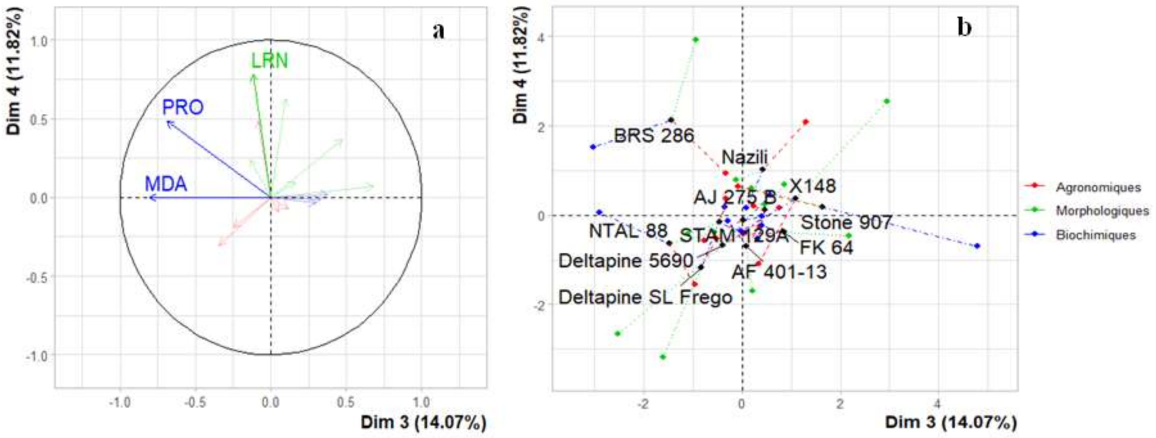

Figure 3. Behavior of variables and individuals in the Dim3-Dim4 plane. a- Correlation circle of variables; b- Projection of individuals in the Dim3-Dim4 plane-PRO: Proline content; MDA: Malondialdehyde content; LRN: Lateral roots number

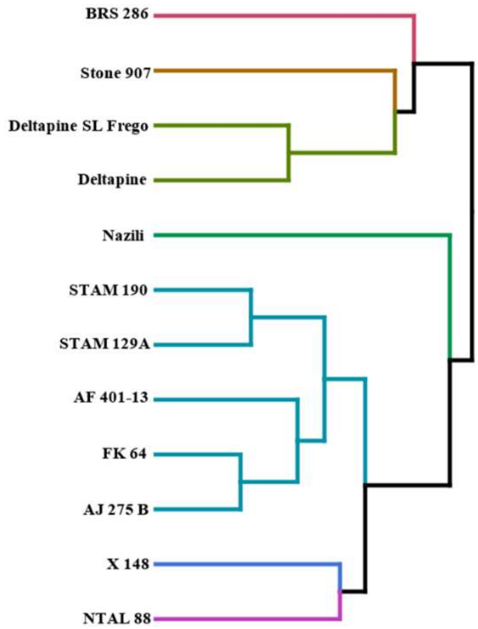

Figure 4. Grouping of varieties according to their similarity by the Hierarchical Ascending Classification Varieties with the same color branches are of the same class

Relationship between the different groups of variables: The graph of the groups of variables (Figure 1) shows that in both planes (Dim1-Dim2 and Dim3-Dim4), the groups of variables have been moved away from each other, so they are not related. Therefore, they do not describe the different individuals studied similarly.

Relationships between individual variables and grouping of individuals: The first dimension was significantly correlated with the (i) biochemical variables: Chla (0.88; $p < 0.001$), ChlT (0.87; $p < 0.001$), Chlb (0.82; $p < 0.01$); (ii) agronomical variables: NSB (0.74; $p < 0.01$), WB (0.74; $p < 0.01$), SCP (0.72; $p < 0.01$), FBOD (0.70; $p < 0.05$) and (iii) morphological variables: FB (-0.58; $p < 0.05$) (Figure 2). The second dimension was correlated with the (i) morphological variables: PH (0.83; $p < 0.001$), VB (0.75; $p < 0.01$), and FBB (-0.69; $p < 0.05$). The projection of individuals in the Dim1-Dim2 plane shows that (i) the variety STONE 907 was characterized by high chlorophyll contents, (ii) the variety Nazili was characterized by a low value of the variables WB and NSB and a high value of FBB (iii) variety X148 was characterized by the high value of PH and VB variables, (iv) variety NTAL88 was characterized by the high value of PH and VB variables, (v) varieties Deltapine 5690 and Deltapine SL Frego had low values of PH, FFAD, and VB.

The third dimension significantly correlated with the variables MDA (-0.81; $p < 0.05$), PRO (-0.69; $p < 0.05$), and FB (0.68; $p < 0.05$), and the fourth dimension correlated with the variable LRN (0.73; $p < 0.01$) (Figure 3). The projection of individuals in the Dim3-Dim4 plane shows that (i) variety STONE 907 (ctr=25.91%) had low MDA and PRO content, (ii) variety NTAL 88 had high MDA and PRO contents, (iii) variety BRS 286 had high values of PRO, and SRN variables and (iv) varieties Deltapine SL Frego and Deltapine 5690 had low values of SRN. The hierarchical ascending classification made it possible to group the varieties into seven classes (Figure 4). Each class was characterized by variables with values higher or lower than the general average at the 5% threshold. Class 1: characterized by the variety with a high value for the variables PRO (758.30 ± 78.1 mg/g MF) and SRN (41.67 ± 5.71 jas). This class consists of the variety BRS 286. Class 2: characterized by the variety with a high value for the variables Chlb (0.16 ± 0.03 mg/g MF), ChlT (0.39 ± 0.08 mg/g MF), and Chla (0.23 ± 0.05 mg/g MF). This class consists of the variety STONE 907. Class 3: characterized by varieties with a low value for the variables PH (73.50 cm ± 1.17 cm) and VB (3.16 ± 0.16 jas). It consists of the varieties Deltapine 5690 and Deltatpine SL Frego ; Class 4: characterized by the variety with a high value of FBB (11.00 ± 1.57) and a low value of the variables WB (2.95 g ± 0.6 g) and SNB (20.33 ± 3.72). It consists of a variety Nazili; Class 5: characterized by varieties whose values do not differ significantly from the mean. This class was composed of the varieties STAM 129A, STAM 190, AF 401-13, FK 64, and AJ 275B. Class 6: is characterized by a variety with a high MRL value (53.33 ± 7.40 cm). It consists of the variety X 148. Class 7: is characterized by the variety with high values for the variables MDA (3.77 ± 0.32 mg/g MF) and FFAD (53.00 ± 1.72 jas). This class is composed of the variety NTAL 88.

DISCUSSION

The results of the present study show that the water deficit caused agro-morphologically, a decrease in seed cotton production, aerial and root biomass, and the number of vegetative branches bolls. Many studies have found similar results (Pettigrew, 2004; Zare *et al*., 2014; Sezener *et al*., 2015; Hassan *et al*., 2018). The reduction of vegetative branch bolls number under water deficit conditions shows that the contribution of VB to seed cotton yield is very random. Dessauw and Hau (1997) showed that, in the cotton breeding program, the reduction of VB is considered a selection criterion for creating variety with stable seed cotton yield in time and space. The results showed, on the other hand, that the water deficit increased the average boll weight. Since the stressed plants lost many bolls, the tiny energy mobilized was used to fill the remaining bolls. Biochemically, it was noted that the deficit during flowering decreases the concentration of chlorophylls in cotton leaves. This decrease is due to the destruction of pigments and the instability of the pigment-protein complex (Levitt, 1980). Many studies have shown that water deficit reduces chlorophyll levels in various crops (Pilon, 2015; Hassan *et al*., 2018; Bokobana *et al*., 2019). Chlorophyll is a pigment responsible for the plant's green color, which is involved in photosynthesis. Therefore, the decrease of its content in the plant during water deficit leads to a decrease in photosynthetic activities and a decrease in carbohydrate manufacturing, thus, leading to a decrease in production in quantity and quality (Anjum *et al*., 2003). The variety that manages to maintain a high chlorophyll content during a water deficit will be able to maintain its photosynthetic activities and, consequently, good production of seed cotton. That is the case of the variety STONE 907 in this study. Sun *et al*. (2021) found that among the criteria for selecting water deficit-resistant cotton, the chlorophyll content of the leaves is essential. Many researchers have used this criterion to determine the tolerance level of a crop under water deficit conditions (Pilon, 2015; Hassan *et al*., 2018).

Resistance mechanisms varied from one variety to another. Some varieties resisted water deficit by increasing the primary root length (X148) or the lateral root number (BRS286). Many scientific works have proved that the root system plays a significant role in plant resistance to water deficit (Loka *et al*., 2011; Comas *et al*., 2013; Uhlla *et al*., 2017; Mahmood *et al*., 2020). Under water deficit conditions, the root allows the plant to avoid dehydration by exploring the deepest soil horizons to increase water availability for the plant. In several crops such as maize, cowpea, rainfed rice, and soybean, Rauf *et al*. (2016) reported that a long and vigorous root helps to maintain yield under water deficit conditions. According to Mahmood *et al*. (2020), a long and dense root system is a desirable trait for better adaptation to water deficit in plants. Similarly, some varieties resisted the deficit by increasing their chlorophyll content (STONE 907), proline content (BRS286), and boll retention (Nazili) or by decreasing the MDA content. For the susceptible varieties, the main characteristics are high MDA content (NTAL 88) and a low lateral root number (Deltatpine SL Frego). In contrast to the results of the evaluation under field conditions (Koffi *et al*., 2021), the present results show that BRS 286 is tolerant to water stress. Indeed, we observed that BRS286 increased its proline content and lateral root number under water stress conditions. Rodriguez *et al*. (2016) found in Brazil that the variety BRS 286 is tolerant to water deficit during flowering due to its ability to synthesize proline. Many studies have proved that proline accumulation is one of the mechanisms developed by the plant to maintain its osmotic balance at the cellular level under water deficit conditions (Maury *et al*., 2011). Proline is an amino acid involved in osmoregulation and the protection of the cell membrane against oxidative stress. Its accumulation during water deficit occurs through the expression of the pyrroline-5-carboxylate synthetase (P-5-CS) gene and the simultaneous repression of the proline dehydrogenase (ProDH) gene (Yoshiba *et al*., 1997). Hassan *et al*. (2018) reported that the resistant cotton variety produced more proline under water deficit conditions than the susceptible varieties. MDA is the end product of membrane lipid peroxidation. Many researchers use it as an indicator of plant tolerance to various abiotic stresses (Bokobana *et al*., 2019; Ladi *et al*., 2020; Gnofam *et al*., 2021). Some studies have shown that MDA increases in plants under water deficit stress; however, this increase is lower in resistant than susceptible varieties.

CONCLUSION

The deficit decreases the seed cotton production per plant, aerial and root biomass, vegetative branches boll number, root volume, and chlorophyll content. However, the average boll weight increased under the water deficit. We observe high variability under water deficit conditions for chlorophyll, total protein, and malondialdehyde content, number of vegetative and fruiting branches, number of fruiting branches bolls, plant height, main stem circumference, lateral root number, and the primary root length. The water deficit tolerant varieties were characterized by the increase in the primary root length (X148), the chlorophyll content (STONE 907), the lateral root number, and proline content (BRS 286), and the decrease of the MDA content (STONE 907). High MDA content (NTALL88) and a low lateral root number (Deltatpine SL Frego) characterized susceptible varieties. STONE 907, BRS286, and X148 can be used as progenitors in crossing programs to improve the water deficit resistance of cotton varieties grown in Togo and PR-PICA countries.

Author Contribution: All authors contributed equally.

Conflict of Interest: There is no conflict of interest in this case report.

Acknowledgements

The authors would like to thank the Togolese new cotton society (NSCT) for sponsoring this research. Also, we thank the team of the Biosecurity et Biosafety Laboratory of Togolese Agronomic research Institute (ITRA) and the plant physiology et biotechnology lab of the Science faculty of the University of Lome (FDS/UL), for providing us the lab materials for the biochemical parameters analysis.

List of abbreviations

AB: Aerial biomass,
Chla: Chlorophyll a concentration,
Chlb: Chlorophyll b concentration,
ChlT: Total chlorophyll concentration,
ETM: Normal water regime,
FB: Fruiting branches number,
FBB: Fruiting branches bolls number,
FBOD: First boll opening date,
FFAD: First flower appearance date,
MDA: Malondialdehyde content,
MFA: Multiple factor analysis,
PRL: Primary root length,
NSB: Number of seeds per boll,
PH: Plant height,
PRO: Leaf proline content,
RB: Root biomass,
RD: Relative difference,
RV: Root volume,
SCP: Seed cotton production per plant,
MSC: Main stem circumference,
LRN: Lateral root number,
STR: 30-day water deficit induced during the flowering phase,
TAW: Total available soil water in mm.cm-1,

TP: Total leaf protein,
VB: Vegetative branches number,
VBB: Vegetative branches bolls number,
WB: Average weight of the bolls.

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