



DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v22n3p176-182>

Physiological, biochemical and productive changes in sesame genotypes subjected to different rates of water replenishment

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Key words:

Sesamum indicum L.
evapotranspiration
antioxidant enzymes

ABSTRACT

The sesame crop has stood out due to the high nutritional content of its seeds, in addition to being able to be cultivated in the tropical and subtropical regions such as Northeast Brazil. Thus, it is necessary to identify the physiological, biochemical and productive changes related to the tolerance to stress. Objective of this study was to evaluate the physiological, biochemical and productive aspects of sesame genotypes as a function of different rates of water replenishment. The experiment was carried out at the Embrapa Cotton Experimental Unit, located in the municipality of Barbalha-CE, Brazil, under field conditions. The experiment was carried out in a randomized complete block design, with treatments in a factorial scheme (4 × 6), corresponding to four irrigation depths (40, 70, 100 and 130% ETo) and six sesame genotypes (G1 = T3-EGSGO3; G2 = T7-EGSGO7; G3 = T5-EGSGO5; G4 = T2-EGSGO2; G5 = T6-EGSGO6; G6 = T4-EGSGO4), with three replicates. Data corresponding to the following variables were collected: leaf area, photosynthetic pigments, relative water content in leaf, electrolyte leakage, catalase, peroxidase, yield and oil content. The genotypes did not differ statistically and there were differences in the variables between the water replacement rates. Increments in growth and, consequently, in production, enzymatic activity, oil content and maximum production potential were observed with water depths between 75 and 90% ETo. Contents of chlorophyll a and b, total chlorophyll and carotenoids, were increased with the application of 80% ETo.

Palavras-chave:

Sesamum indicum L.
evapotranspiração
enzimas antioxidantes

Alterações fisiológicas, bioquímicas e produtiva em genótipos de gergelim submetidos a diferentes taxas de reposição hídrica

RESUMO

O cultivo de gergelim vem se destacando devido ao alto teor nutricional de suas sementes, além de poder ser cultivado nas regiões tropicais e subtropicais como o Nordeste brasileiro, sendo necessária a identificação das alterações fisiológicas, bioquímicas e produtivas relacionadas com a tolerância ao estresse. Objetivou-se avaliar o comportamento fisiológico, bioquímico e produtivo de genótipos de gergelim em função de diferentes taxas de reposições hídricas. O experimento foi realizado na Unidade Experimental da Embrapa Algodão, localizada no Município de Barbalha, CE, sob condições de campo. O delineamento adotado foi em blocos casualizados, com tratamentos em esquema fatorial (4 × 6), correspondendo a quatro lâminas de irrigação (40; 70; 100 e 130% da ETo) e seis genótipos de gergelim (G1 = T3-EGSGO3; G2 = T7-EGSGO7; G3 = T5-EGSGO5; G4 = T2-EGSGO2; G5 = T6-EGSGO6; G6 = T4-EGSGO4) com três repetições. Foram coletados dados correspondentes às variáveis: área foliar, pigmentos fotossintéticos, teor de relativo de água na folha, extravasamento de eletrólitos, catalase, peroxidase, produtividade e teor de óleo. Os genótipos de gergelim não diferiram estatisticamente, no entanto houve diferenças entre as reposições hídricas com um aumento no crescimento e consequentemente, na produção, atividade enzimática, teor de óleo e o potencial máximo da produção com a aplicação de lâminas entre 75 e 90% da ETo. O conteúdo de clorofila a e b, total e carotenoides foram incrementados com a aplicação de 80% da ETo.



INTRODUCTION

In Northeast Brazil, where rainfall scarcity and/or poor distribution prevails, water deficit is a common and limiting condition to agricultural production, which makes the cultivation in the rainy period (rainfed) a highly vulnerable alternative. Nevertheless, in these regions, irrigation can be used to supplement rainfall, which leads to improvement in the growth, development and yield of crops. According to Bernardo et al. (2009), water replacement in the soil makes the production viable, regularizes and completes the use of soil, and can increase crop production. Additionally, these authors highlight that cultivation success depends on supplemental irrigation during the critical period of the crop.

Sesame (*Sesamum indicum* L.) is one of the first oilseed crops used by humans, occupying the ninth position among the most cultivated in the world (Bezerra et al., 2010; Perin et al., 2010; Mesquita et al., 2013; Pinto et al., 2014; Silva et al., 2014). Its yield depends on various climatic factors, such as temperatures between 25 and 30 °C, altitudes below 500 m and rainfall from 500 to 650 mm (Ariél et al., 2009). The sesame crop is resistant to drought and can produce with 300 mm of water well distributed along the cycle (Bernardo et al., 2009), which allows its cultivation in arid or semi-arid zones and in periods of scarce rainfall.

In the last years, many studies have been carried out using sesame, usually involving spacing, plant density and irrigation (Pinto et al., 2014). However, no studies were found on the determination of physiological and biochemical variables in genotypes of sesame under different water depths.

In this context, this study aimed to evaluate the physiological, biochemical and productive behavior of sesame genotypes as a function of different rates of water replacement.

MATERIAL AND METHODS

The experiment was carried out from September to December 2013 at the Embrapa Cotton Experimental Unit, located in the municipality of Barbalha, CE, Brazil (7° 19' S; 39° 18' W; 415.74 m). The climate of the region is Aw', according to Köppen's classification, characterized as rainy tropical, very hot, with rainfalls predominantly in the summer and winter. The region has mean rainfall of 1523 mm, mean air temperature of 26.9 °C and relative humidity of 69% (Data provided by the Embrapa Cotton Meteorological Station). Along the experimental period, temperature, relative humidity, wind speed and rainfall data (Table 1) were collected at the Embrapa Cotton Meteorological Station, in Barbalha-CE.

The experiment was conducted in a clayey soil, sampled until 100 cm deep and classified as clay loam Fluvisol (RY),

Table 1. Mean temperature (Tm), relative air humidity (RH), wind speed (WS) and rainfall (P) recorded along the experimental period

Month	Tm (°C)	RH (%)	WS (m s ⁻¹)	P (mm)
September	37.83	67	2.38	0.76
October	37.94	66	2.62	4.82
November	38.45	63	2.62	3.80
December	38.88	63	2.66	3.81

whose textural and physical characterizations were performed at the Irrigation and Salinity Laboratory of the Academic Unit of Agricultural Engineering of the Federal University of Campina Grande. For soil chemical characterization, according to the methodology of Carmo et al. (2000), before installing the experiments, samples were collected in the 0-0.20 m layer using a soil sampler, and taken to the Soil and Plant Nutrition Laboratory of the Embrapa Cotton, for analysis, and the following values were obtained: pH (H₂O) = 6.8; P = 5.4 mg dm⁻³; K⁺ = 0.14 cmol_c dm⁻³; Na⁺ = 0.28 cmol_c dm⁻³; H⁺ + AL³⁺ = 0.33 cmol_c dm⁻³; Al³⁺ = Not detected; Ca²⁺ = 9.53 cmol_c dm⁻³; Mg²⁺ = 4.92 cmol_c dm⁻³; sum of cations = 14.87 cmol_c dm⁻³ and organic matter = 12.3 g kg⁻¹.

The experimental design was randomized complete blocks, with treatments in factorial scheme (4 × 6), corresponding to four irrigation depths (40; 70; 100 and 130% ETo) and six sesame genotypes (G1 = T3-EGSGO3; G2 = T7-EGSGO7; G3 = T5-EGSGO5; G4 = T2-EGSGO2; G5 = T6-EGSGO6; G6 = T4-EGSGO4), totaling 24 treatments, with three replicates. The experimental plot comprised four rows, and the two central rows were used for evaluations.

Tillage in the experimental area consisted in one plowing and four cross harrowing procedures, using a leveling harrow. Then, basal fertilization was performed based on soil chemical analysis, using a dose equivalent to 300 kg ha⁻¹ of monoammonium phosphate - MAP (11% N and 46% P₂O₅). Top-dressing fertilizations were performed after thinning or 45 days after emergence (DAE), applying 100 and 50 kg ha⁻¹ of urea (45% N) and potassium chloride (60% K₂O), respectively, in furrows opened parallel to the lateral rows.

Irrigation management was performed using the water depth principle, based on the replacement of reference evapotranspiration (ETo) for two consecutive days. ETo was estimated using the Penman-Monteith equation standardized by FAO (Pereira et al., 1997), and climate data were daily collected at the Embrapa Cotton Meteorological Station in Barbalha-CE. Irrigation depth (Li) and irrigation time (Ti) were calculated according to Mantovani et al. (2006).

The sesame genotypes used were white and cream-colored, all developed by the Brazilian Agricultural Research Corporation (Embrapa Cotton). Their main characteristics include: cycle of 90-95 days, beginning of flowering at 35 days, mean yield of 1,000 kg ha⁻¹, oil content between 50 and 53% and resistance to the main diseases of the crop (Ariél, et al., 2009).

All evaluations, except of production, were carried out at 90 days after emergence (DAE). Total leaf area (cm²) was determined by measuring the length and width of leaflets and using the equation proposed by Silva et al. (2002).

Leaf relative water content was measured according to Smart & Bingham (1974). Cell membrane integrity evaluation, based on electrolyte leakage, was expressed as percentage of conductivity in relation to the total conductivity. Chlorophylls *a*, *b* and total, and carotenoids were extracted using the solvent dimethyl sulfoxide (Hiscox & Israelstam, 1979).

At 50 DAE, leaf samples were collected to determine enzymatic activity: catalase (CAT) according to Haver & McHale (1987); and peroxidase (POD), based on peroxide consumption (Dann & Deverall, 2000).

At 95 DAE, harvest was done manually by cutting the whole plant at soil level. Then, plants were air-dried for seven days to facilitate the removal of seeds from the capsules. Yield was estimated based on the production obtained in the evaluation area of the experimental plot.

Oil content in the seeds was quantified by nuclear magnetic resonance (NMR), based on non-destructive tests, in which the value found results from the magnetic field scanning of the sample (Oxford Instruments, 2007).

Data of the response variables were subjected to the analysis of variance by F test (Fisher) at 0.05 probability level. Quantitative factors (irrigation depth) were subjected to polynomial regression analyses, selecting the model of highest significance in the F test (Ferreira, 2008). Analyses were carried out using the statistical programs Sisvar 5.3 and Table Curve 2D.

RESULTS AND DISCUSSION

Table 2 shows the analysis of variance summary for the growth physiological, biochemical and production variables. Irrigation depths influenced leaf area (LA), total leaf phytomass (TLP), chlorophyll *a* (Chla), chlorophyll *b* (Chlb), carotenoids (CAR), total chlorophyll (Chlt), catalase (CAT), peroxidase (POD), yield (Y), oil content (OC), electrolyte leakage (EL), relative water content (RWC), at 0.01 probability level. For the interaction irrigation depth x genotypes, there was no significance.

Sesame leaf area (LA) and total leaf phytomass (TLP) values fitted to a quadratic polynomial model (Figures 1 A and 1B). Maximum points of LA (63.29 cm²) and TLP (7.62 g) were estimated at water replacement rates equivalent to 85.75 and 86.07% ETo, respectively. Increased water replacement in the soil resulted in decreases of 48.81 and 56.34% in LA and TLP, concomitantly, reaching values statistically similar to those found with water replacement of 40% ETo (Figure 1A and 1B). This behavior demonstrates sesame sensitivity to both deficit and excess of water in the soil.

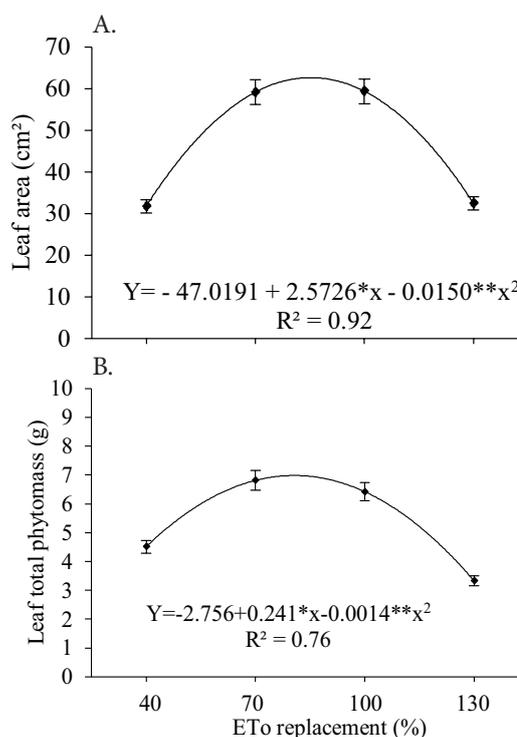


Figure 1. Leaf area (A) and total leaf phytomass (B) of sesame genotypes subjected to water replacement at 90 days after sowing

Similar response was found by Silva et al. (2002), who observed that leaf area at 90 DAE was drastically affected by irrigation depths lower than 250 mm and higher than 1000 mm, and maximum leaf area was obtained with the application of 698.88 mm. These results corroborate the hypothesis that sesame is sensitive to water stress.

Plant water relationships have been widely investigated under water deficit conditions, and leaf water content is accepted as an indicator of plant water conditions. In the sesame genotypes evaluated here, the maximum estimated relative water content (RWC) (22.81%) was found with estimated water replacement of 52.16% ETo (Figure 2A), which represents an increment of 14.32% in comparison to the value found with water replacement of 40% ETo.

Table 2. Summary of analysis of variance for leaf area (LA), total leaf phytomass (TLP), chlorophyll *a* (Chla), chlorophyll *b* (Chlb), carotenoid (CAR), total chlorophyll (Chlt), catalase (CAT), peroxidase (POD), yield (Y), oil content (OC), electrolyte leakage (EL), relative water content (RWC), in sesame genotypes subjected to different rates of water replacement at 90 days after sowing

SV	DF	Mean square						
		LA	TLP	Chla	Chlb	CAR	Chlt	
Block	2	0.7379 ^{ns}	5.418*	130782.32**	136.99 ^{ns}	7473.16**	1150.06 ^{ns}	
Irrig. Depth (L)	3	4804.7947**	67.543**	365794.53**	3082.73**	10221.40**	142850.5**	
Linear	1	4.3212 ^{ns}	4.534 ^{ns}	4810.74 ^{ns}	16.17**	5150.13 ^{ns}	82576.9**	
Quadratic	1	13280.025**	159.965**	753471.70**	6002.17**	14314.25**	205426.2**	
Genotypes (G)	5	112.1498 ^{ns}	1.034 ^{ns}	9377.03 ^{ns}	144.85 ^{ns}	171.34 ^{ns}	2705.31 ^{ns}	
Int. L X G	15	22.5634 ^{ns}	5.339 ^{ns}	6087.27 ^{ns}	325.36 ^{ns}	496.07 ^{ns}	1410.36 ^{ns}	
Residual	46	59.0974	1.4947	5845.59	202.90	1416.75	1764.7	
CV (%)		16.84	23.85	22.77	30.13	22.82	15.75	
		CAT	POD	Y	OC	EL	RWC	
Block	2	18.07*	80.12 ^{ns} **	85689.758*	131.801 ^{ns}	131.14*	231.53*	
Irrig. Depth (L)	3	117.25**	3781.36**	578575.204**	18969.6222**	5012.06**	133.46*	
Linear	1	5.15 ^{ns}	587.45**	14357.775 ^{ns}	7.140 ^{ns}	515.34**	248.56 ^{ns}	
Quadratic	1	223.03**	9637.53 ^{ns}	727549.888**	6046.365**	6134.68**	30.22**	
Genotypes (G)	5	12.77 ^{ns}	350.91 ^{ns}	12943.420 ^{ns}	143.614 ^{ns}	101.98*	22.40 ^{ns}	
Int. L X G	15	3.71 ^{ns}	185.67 ^{ns}	11122.65 ^{ns}	160.711 ^{ns}	10.12 ^{ns}	27.45 ^{ns}	
Residual	46	4.87*	244.94	23117.187	86.403	39.79	54.59	
CV (%)		30.42	33.92	35.68	20.87	15.49	11.52	

SV- Sources of variation; CV- Coefficient of variation; DF – Degrees of freedom; Int.- Interaction; **, Significant at 0.01 probability level; ns – not significant by F test (p<0.05)

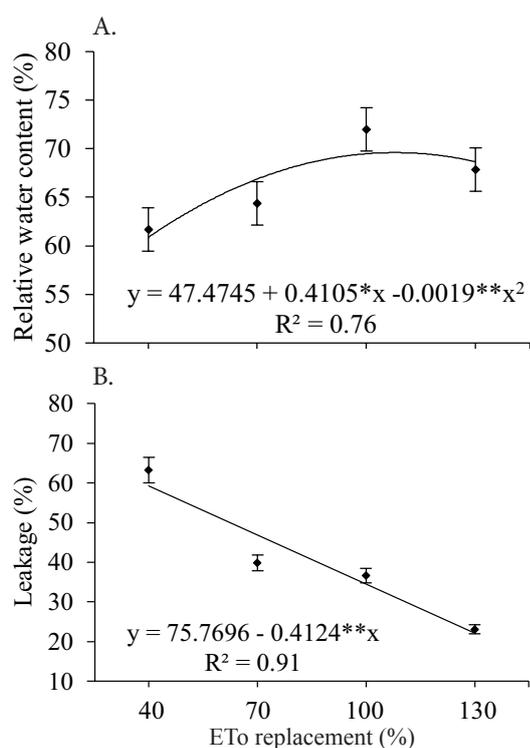


Figure 2. Relative water content (A) and electrolyte leakage (B) in sesame genotypes subjected to water replacement at 90 days after sowing

The reductions in RWC observed here were lower than those reported by Pinto et al. (2014), who obtained mean decrease of 27% by exposing sesame plants to successive cycles of water deficit. These results reinforce the hypothesis that continuous water availability, even in small amount (40% ETo), induces greater maintenance of water content in the leaves, which may influence the functioning of the photosynthetic apparatus.

The increase in electrolyte leakage as soil water content decreased can be attributed to a consequence of the limitation in photosynthesis imposed by the water deficit, which results in disruption of membrane integrity, triggered by the overproduction of reactive oxygen species (ROS). High contents of these compounds can reduce the activity of enzymes, cause peroxidation of lipids, oxidation of proteins and, in the most advanced stage, kill the cell (Sharma et al., 2012).

For electrolyte leakage (EL), the data of the sesame genotypes fitted to a decreasing linear model and maximum estimated value (59.27%) was found with 40% ETo replacement (Figure 2B). It is worth noting that water replacement variation from 40 to 130% ETo resulted in reduction of 62.61% in the EL of the sesame genotypes.

Water deficit is closely related to the loss of pigments and, consequently, to the reduction in the photosynthetic and productive capacity of plants. Hence, analysis of photosynthetic pigments is essential to evaluate the health and integrity of internal cell apparatus during photosynthesis (Taiz & Zeiger, 2009; Rigon et al., 2012), as well as an indicator of the damages suffered.

In the sesame plants evaluated, chlorophyll contents (*a*, *b* and total) fitted to a quadratic polynomial model (Figure 3A, B and C) and their maximum estimated values (499.74, 66.78,

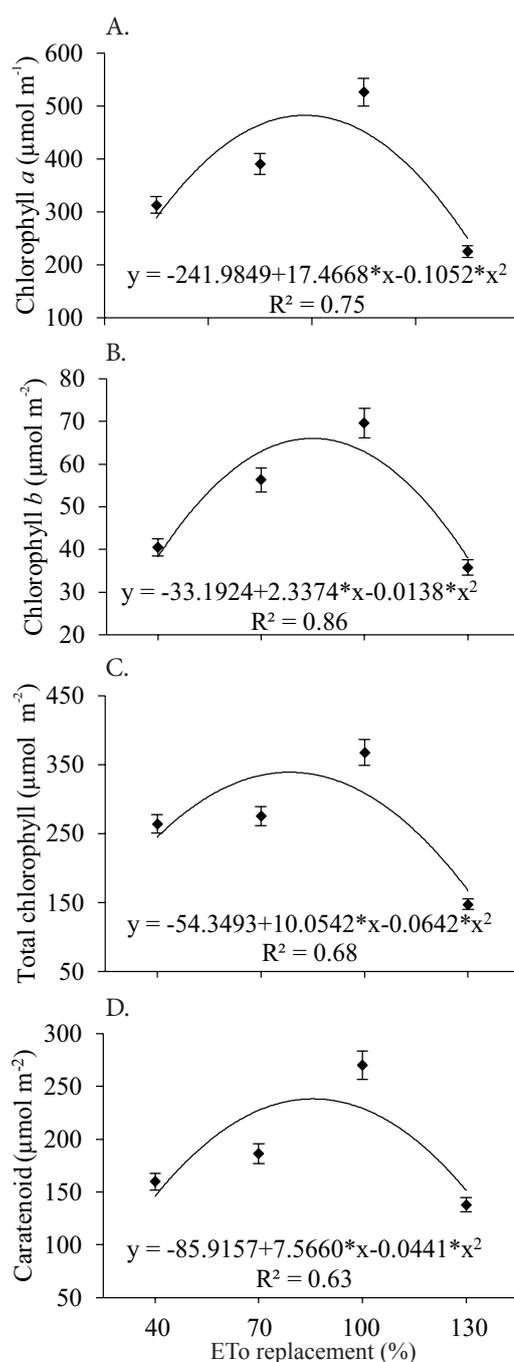


Figure 3. Contents of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in sesame genotypes subjected to water replacement at 90 days after sowing

and $339.29 \mu\text{mol m}^{-2}$) were found at estimated water depths of 83.97, 84.69 and 78.30% ETo, respectively. Water replacement above these values caused reduction in chlorophyll contents, which reached values lower than those found with 40% ETo replacement.

Chlorophyll is one of the main factors related to plant photosynthetic efficiency and, consequently, to growth and adaptability to different environments and conditions caused by the various types of stress. Chlorophyll *b*, considered as accessory pigment, is a biochemical compound present in the chloroplasts that help light absorption. Its production is directly associated with chlorophyll *a* production (Silva et al., 2002), and is more intense when plants are exposed to stress conditions.

Therefore, the lower values found with water depth of 40% ETo may be an indication of absence of more severe stress on the plants, compared with the values found with 130% ETo replacement. Such behavior may indicate that sesame plants are more susceptible to the stress caused by excess of water than the one caused by its absence, corroborating the claim of Arriel et al. (2009), who point out that the crop, in general, is resistant to drought and suitable for cultivation in arid and semi-arid zones and in periods of scarce rainfall.

For the content of carotenoids, maximum estimated value ($238.60 \mu\text{mol m}^{-2}$) was obtained with estimated water depth of 85.78% ETo (Figure 3D). Comparing the maximum content of carotenoids with the values found at water depths of 40 and 130% ETo, there was an increase of 63.23% as water depth varied from 40 to 85.78% ETo, followed by a reduction of 36.14% as water depth increased to 130% ETo.

Determining the content of carotenoids in plants subjected to some sort of stress is very important, due to their role in the dissipation of the energy intercepted. These pigments act as photoprotectors, deactivating singlet and triplet oxygen by absorbing the energy present in these compounds during their formation, which prevents oxidative damages to plant cells (Engel & Poggiani, 1991; Taiz & Zeiger, 2009; Brito et al., 2011; Rigon et al., 2012).

In this context, it can be inferred that plants with lower contents of this pigment would be more subject to damages caused by the excess of light to the photosystem. Nevertheless, reduction in carotenoids combined with low content of chlorophyll *a* in the leaves of plants subjected to both absence and excess of water may contribute to increasing the injuries to the photosynthetic mechanism, leading to limitations in growth, development and production.

Plants protect their cells and subcellular compartments from cytotoxic effects caused by ROS by using antioxidant enzymes, such as catalase and peroxidase. In natural environment, antioxidative responses to abiotic variations are induced by similar mechanisms, which result in adaptations to drier or more humid periods, to days with higher or lower light incidence, as well as to high and low temperatures, since these factors lead to the increase in ROS contents and, consequently, stimulate likewise antioxidant activity (Sharma et al., 2012).

In the present study, water replacements below 80% and above 100% ETo reduced the antioxidant enzymatic activity. For catalase, lower activity was observed with the application of 40% ETo; however, the increment in water availability increased the activity of this enzyme until its maximum point ($10.11 \mu\text{g mL}^{-1}$), estimated at the water depth corresponding to 87.47% ETo (Figure 4A). According to Sharma et al. (2012), occurrence of environmental stress may increase or decrease catalase activity, depending on the intensity, duration and type of stress.

Differently from what was observed, the lowest activity of peroxidase was observed with application of 130% ETo. Maximum estimated activity ($66.24 \mu\text{g mL}^{-1}$) for peroxidase was found at estimated water depth of 79.24% ETo (Figure 4B). Such reduction in the activity of the enzymes in plants subjected to water stress conditions may be an indication of

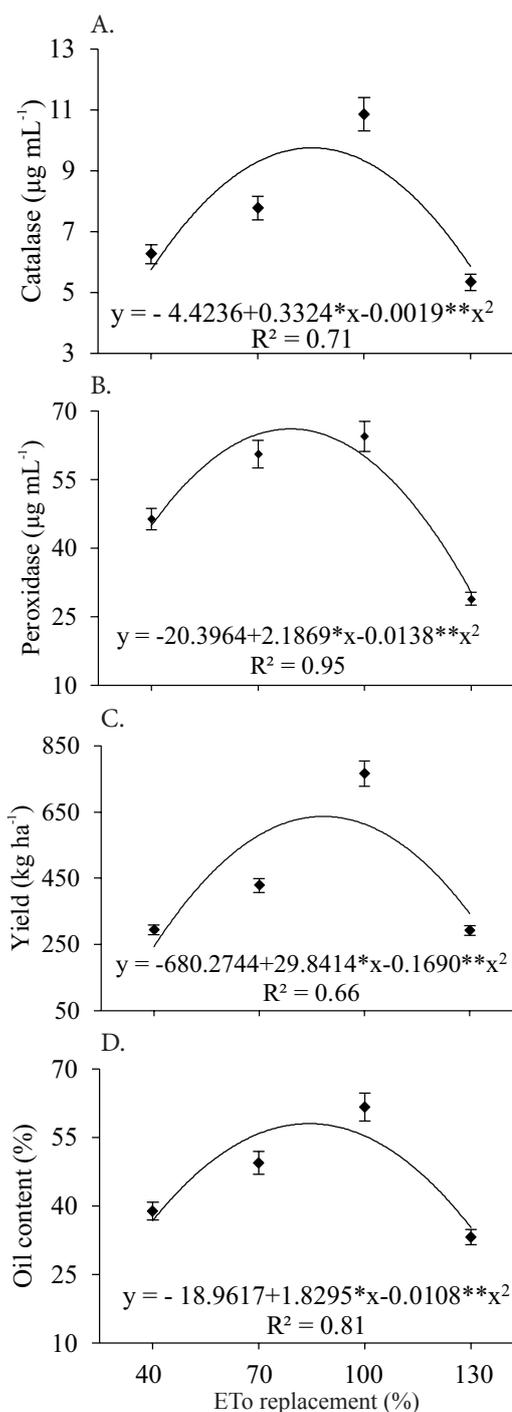


Figure 4. Antioxidant activity of catalase (A), peroxidase (B) at 50 days after sowing and grain yield (C) and oil content (D) in the seeds of sesame genotypes subjected to water replacement at 95 days after sowing

non-adaptation to the stressful environment, with effect on crop yield.

Regarding sesame yield (Figure 4C), the data fitted best to a quadratic polynomial model in the regression analysis, in which the application of 40% ETo led to the lowest mean yield estimated ($242.92 \text{ kg ha}^{-1}$), even lower than the means of the world ($481.40 \text{ kg ha}^{-1}$) and of the country ($600.00 \text{ kg ha}^{-1}$), with plantations under rainfed conditions (Silva et al., 2002).

In contrast, from the water depth of 40% ETo on, there was a sharp increase in yield, reaching maximum of $637.19 \text{ kg ha}^{-1}$ when irrigated with estimated water depth of 88.29% ETo, and

decreasing by 46.16% until the value of 343.01 kg ha⁻¹ with the application of 130% ETo. Maximum yields found with intermediate water depths may be directly associated with the improvements observed in physiological and biochemical processes, such as the water content in the leaves.

In addition, yield reduction is directly related to the water stress conditions, because plants use the stomatal closure mechanism to protect themselves from water losses through transpiration, leading to a reduction in the photosynthetic rate (Taiz & Zeiger, 2009).

Oil content in the seeds increased with the increment in water replacement until the estimated water depth of 84.70% ETo, where maximum oil content was obtained (58.52%), 36.94% higher than that found with application of the lowest irrigation depth (40%) (Figure 4D). Nonetheless, increment in water depth above 84.70% caused mean reduction of 32.75% in the oil content of the seeds, reaching minimum value of 36.35%.

It is known that sesame oil is rich in unsaturated fatty acids, such as oleic (47%) and linoleic (41%), and has various secondary constituents that are very important to define its chemical properties, such as sesamol, sesamin and sesamol. Sesamol, with its antioxidant properties, promotes high chemical stability in the oil, preventing rancidification. Sesame oil has the highest resistance to oxidation among the other vegetable oils (Beltrão et al., 1994). In this context, sesame oil content becomes one of the most important variables to evaluate the feasibility of the crop from the agronomic point of view.

CONCLUSIONS

1. Sesame genotypes did not differ statistically and there were differences in the variables between the water replenishment rates. Increments in growth and, consequently, in production, enzymatic activity, oil content and maximum production potential were found with water depths between 75 and 90% ETo.

2. Contents of pigments, chlorophyll *a*, *b*, total and carotenoids, increased with the application of 80% ETo.

ACKNOWLEDGMENTS

To the Coordination for the Improvement of Higher Education Personnel (CAPES) and the National Postdoctoral Program (PNPD) for granting the scholarships and financial support. To the Brazilian Agricultural Research Corporation (Embrapa Cotton) for providing the structure needed to conduct this study. In special, to Dr. Napoleão Esberard de Macêdo Beltrão (*In memoriam*) for his idealization and contributions during all the experiment.

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