Pedosphere **22**(6): 785–794, 2012
ISSN 1002-0160/CN 32-1315/P

Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Effect of Combined Use of Brackish Water and Nitrogen Fertilizer on Biomass and Sugar Yield of Sweet Sorghum*[1]

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(Received April 18, 2012; revised August 31, 2012)

ABSTRACT

Soil salinization and non-point source pollution are among the most important and widespread environmental problems in European Mediterranean regions. Sweet sorghum (*Sorghum bicolor* (L.) Moench var. *saccharatum*) is a moderate to high salinity tolerant crop with low water and nutrient needs, seen as an alternative to grow in the water scarce regions. A three-year multifactorial study was conducted in southern Portugal to evaluate the combined effects of saline water and nitrogen application on the dry biomass (total, stems, and leaves), sugar content (total reducing sugars and sucrose contents), and sugar yield (here defined as the product of total reducing sugars and stems dry biomass) functions of sweet sorghum. Sorghum dry biomass and sugar yield showed diminishing returns for each incremental change of nitrogen. The use of saline irrigation waters also led to yield reduction. Exception was sucrose content which increased with increasing levels of sodium in the soil. Nitrogen need decreased as the amount of sodium applied increased. Stem dry biomass, sucrose content, and sugar yield progressively increased with progress in the experiment. The effect could be attributed to the increase of the amount of irrigation applied throughout the years, thus increasing the leaching fraction which promoted salt leaching from the root zone, reduced the salinity stress, increased plant transpiration, nitrogen uptake and biomass yield.

Key Words: Mediterranean conditions, non-point source pollution, salinity, sweet sorghum, yield functions

Citation: Ramos, T. B., Castanheira, N. L., Gonçalves, M. C., Fernandes, M. L., Januário, M. I., Lourenço, M. E., Pires, F. P. and Martins, J. C. 2012. Effect of combined use of brackish water and nitrogen fertilizer on biomass and sugar yield of sweet sorghum. *Pedosphere*. **22**(6): 785–794.

INTRODUCTION

Human-induced soil salinization, sodification and non-point source pollution are among the most important and widespread environmental problems in agricultural regions with arid, semi-arid, and even dry subhumid conditions. Improving crop productivity by use of water and nutrients more efficiently has been a leading research approach for these water scarce regions (Pereira *et al.*, 2009). Crop yield-water consumption relationships are among the most common techniques used to measure the effects and interactions of water and nutrients on crop yield (Igbadun *et al.*, 2007; Mandal *et al.*, 2010). However, in water scarce regions even saline waters are seen as an important resource to meet food demands (Rhoades *et al.*, 1992; Qadir and Oster, 2004; Pereira *et al.*, 2009). Thus, the quality of the irrigation water and the understanding of the combined effects of saline water and nitrogen application on the yield function are of fundamental importance since it could give answers on how to overcome the soil salinization/sodification process and how to reduce nitrate leaching in those regions (Dinar *et al.*, 1991; Datta *et al.*, 1998).

Besides improving water and nutrients efficiency, the use of crops with less water and nutrient needs and higher salt tolerance has been considered by the scientific community as an alternative to traditional crops in the water scarce regions (Zhao *et al.*, 2009; Vasilakoglou *et al.*, 2011). Sweet sorghum (*Sorghum bicolor* (L.) Moench) is seen as one of the most interesting annual crops to grow in these stressed areas (Lourenço

*[1]Supported by the Foundation for Science and Technology (FCT) of Portugal (Nos. PTDC/AGR-AAM/66004/2006, SFRH/BD/60363/2009 and SFRH/BD/69185/2010).
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et al., 2007; Vasilakoglou *et al.*, 2011). Sweet sorghum has a high drought resistance due to its relatively low water requirements (Mastrorilli *et al.*, 1999) and high water-use efficiency (Mastrorilli *et al.*, 1995; Steduto *et al.*, 1997), and possesses a moderate to high tolerance to salinity (Maas, 1990). Sweet sorghum is furthermore an important alternative source for renewable energy by storing large quantities of non-structural carbohydrates (sucrose, glucose, and fructose) in the stems which can be converted into fuel ethanol (Almodares and Hadi, 2009).

The effects of N fertilizer on sweet sorghum growth and yield have been evaluated for several times in various regions of the world (Wiedenfeld, 1984; Barbanti *et al.*, 2006). The effect of the salinity stress on sweet sorghum have been also presented in literature (Begdullayeva *et al.*, 2007; Almodares *et al.*, 2008a, b; Vasilakoglou *et al.*, 2011). However, studies focusing on the combined effects of saline water and nitrogen application on sweet sorghum have been very limited despite the growing interest for this annual crop throughout vast regions of the world. Thus, the objective of this study was to evaluate the combined effects of saline water and nitrogen application on sweet sorghum (*Sorghum bicolor* (L.) Moench) dry biomass and sugar yield (here defined as the product of total reducing sugars and stems dry biomass) functions under Mediterranean conditions.

MATERIALS AND METHODS

Site description

Field plot experiment was conducted at the Alvalade Experimental Station (37° 56′ 48″ N and 8° 23′ 40″ W) located in the Alentejo region of southern Portugal. The experiment was carried out from 2007 to 2009, always at the same location, on a field with an Eutric Fluvisol. The soil is a clay loam with coarse sand 8.3%, fine sand 52.4%, silt 26.3%, and clay 13.0% by weight; dry bulk density 1.49 g cm^{-3}; the field capacity 31.0%; the wilting point 9.8%; the saturated hydraulic conductivity 14.2 cm d^{-1}; pH (H_2O) 7.2; the average organic matter 20.0 g kg^{-1}; cation exchange capacity 11.67 cmol$_c$ kg^{-1}; electrical conductivity (EC) of the saturation extract 3.04 dS m^{-1}; and at the beginning of the three growing seasons of 2007, 2008 and 2009, exchangeable Na^+ 1.94, 1.53, and 1.84 cmol$_c$ kg^{-1}; NO_3^--N 2.90, 4.60, and 4.85 mg kg^{-1}; and NH_4^+-N 2.17, 0.46, and 0.55 mg kg^{-1}, respectively.

The climate in the Alentejo region is mostly dry sub-humid to semi-arid, with hot dry summers, and mild winters with irregular rainfall. Sweet sorghum potential transpiration (T_p) rates in each field plot were obtained from the daily values of reference evapotranspiration (ET_0), determined with the FAO Penman-Monteith method (Allen *et al.*, 1998; 2005), as follows:

$$ET_c = (K_{cb} + K_e)ET_0 \tag{1}$$

where ET_c is the crop evapotranspiration (L T^{-1}), K_{cb} is the basal crop coefficient, which represents the plant transpiration component, and K_e is the soil evaporation coefficient. Standard sweet sorghum K_{cb} values (Allen *et al.*, 1998) were adjusted for the Alvalade climate, taking into consideration the crop height, wind speed, minimum relative humidity averages for the period under consideration, as well as the effect of the salinity and nitrogen stress on the leaf area index (LAI), using the procedure for non-pristine agricultural vegetation described in Allen *et al.* (1998).

Crop management

Sweet sorghum (*Sorghum bicolor* (L.) Moench var. *saccharatum*) was sown on May 18, 2007, May 15, 2008, and May 15, 2009. The hybrid selected for this experiment was sweet sorghum "Madhura" developed in India. The row spacing used was 0.75 m and the distance between plants was 0.15 m. Soil surface was prepared for seeding using a minimum tillage system. The crop was irrigated with a trickle irrigation system. Irrigation started 15 days after sowing (DAS) in 2007, 30 DAS in 2008, and 20 DAS in 2009. The total amount of water applied was 425, 522, and 546 mm in 2007, 2008, and 2009, respectively. Experimental plots were irrigated three times per week between June and September. Application amounts averaged 15, 16, and 17 mm per irrigation event occurred in 2007, 2008, and 2009, respectively. Nitrogen fertilization (NH_4NO_3) was applied in 4 (2007), 6 (2008), and 3 (2009) irrigation events during the vegetative stage (July). The crop was harvested manually at 132 DAS in 2007, 138 DAS in 2008, and 137 DAS in 2009. From harvest to the following growing season, soil was left with no crop and subjected to atmospheric conditions, namely rainfall leaching.

Experimental design and treatments

The trickle irrigation system was used to mix and deliver synthetic saline irrigation waters, fresh irrigation waters, and fertilizer (NH_4NO_3) to the experimental plots following the irrigation scheme documented in Ramos *et al.* (2009, 2011, 2012). This system consisted of three trickle laterals connected together in order to form a triple joint lateral placed along each crop line.

TABLE I

Irrigation waters blended in each experimental plot during the three crop seasons of of 2007, 2008, 2009

Subgroup		Group											
		I			II			III			IV		
		2007	2008	2009	2007	2008	2009	2007	2008	2009	2007	2008	2009
		mm											
A	Saline water	228.0	184.0	316.0	228.0	184.0	316.0	228.0	184.0	316.0	228.0	184.0	316.0
	Water + NH_4NO_3	19.3	30.0	20.0	12.9	20.0	13.3	6.4	10.0	6.7	0.0	0.0	0.0
	Fresh water	177.7	308.0	210.0	184.1	318.0	216.7	190.6	328.0	223.3	197.0	338.0	230.0
B	Saline water	114.0	92.0	158.0	114.0	92.0	158.0	114.0	92.0	158.0	114.0	92.0	158.0
	Water + NH_4NO_3	19.3	30.0	20.0	12.9	20.0	13.3	6.4	10.0	6.7	0.0	0.0	0.0
	Fresh water	291.7	400.0	368.0	298.1	410.0	374.7	304.6	420.0	381.3	311.0	430.0	388.0
C	Saline water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Water + NH_4NO_3	19.3	30.0	20.0	12.9	20.0	13.3	6.4	10.0	6.7	0.0	0.0	0.0
	Fresh water	405.7	492.0	526.0	412.1	502.0	532.7	418.6	512.0	539.3	425.0	522.0	546.0

The first of the laterals was connected to the salt stock solution (NaCl), while the second one was connected to the N reservoir. The third lateral delivered fresh water and was used to obtain a constant water application rate for each dripping point along the triple joint lateral (24 mm h^{-1}). Gradients of applied salt (Na^+) and nitrogen (N) concentrations were then produced by placing different emitters in each dripping point along the corresponding laterals and varying their discharge rates to obtain various mixtures between the three lines. Table I presents the amounts of saline waters, fresh waters, and waters with nitrogen applied in each experimental plot. The blended amounts thus varied between experimental plots, while the total amount of water applied per irrigation event and per crop season, as well as the quality of the irrigation waters before blending (Table II), remained identical in all experimental plots.

TABLE II

Characteristics of the irrigation waters blended in each experimental plot

Irrigation water	Year	EC_{iw}[a)]	NH_4^+-N	NO_3^--N
		dS m^{-1}	mmol$_c$ L^{-1}	
Fresh water	2007–2009	0.81	0.03	0.15
Saline water	2007	7.60	0.03	0.15
	2008	9.60	0.03	0.15
	2009	10.60	0.03	0.15
Water with nitrogen	2007	9.50	95.0	95.0
	2008	6.77	67.7	67.7
	2009	7.34	73.4	73.4

[a)] Electrical conductivity of the irrigation water.

The experimental field was thus divided into four groups (I–IV), each with three triple joint laterals, establishing an N gradient decreasing from group I to group IV. Each group was then divided into 3 subgroups, A, B and C, each with a surface area of 6.75 m^2 (2.25 m × 3 m), and with the Na^+ gradient decreasing from A to C. The amount of N applied in each group (I–IV) is presented in Table III. The amount of Na^+ applied in the different subgroups (A–C) is presented in Table IV. The dripping points were spaced 1 m apart, with a total of 9 dripping points in each of the 12 experimental plots. Two laterals of fresh water bordered the different groups. Each subgroup area was bordered with earthen ridges, which prevented surface runoff from crossing over during rainfall and irrigation.

TABLE III

Total amount of nitrogen (N) applied in each group plot during the three irrigation seasons of 2007, 2008, 2009

Group	2007	2008	2009
	g m^{-2}		
I	51.4	56.8	41.1
II	34.2	37.9	27.4
III	17.2	19.0	13.7
IV	0.0	0.0	0.0

TABLE IV

Total amount of Na^+ applied in each subgroup during the three irrigation seasons of 2007, 2008, 2009

Subgroup	2007	2008	2009
	g m^{-2}		
A	1 096	1 133	2 166
B	576	599	1 162
C	60	66	158

Biomass and sugar yield determination

Sugar content was determined at milk stage 95 DAS (in 2007), 112 DAS (in 2008), and 110 DAS (in 2009) when the maximum sugar yield was achieved (Almodares and Hadi, 2009). In each experimental plot, four plants were randomly selected, and harvested manually for biomass and sugar content determination. The stems were cut in 0.3 m long pieces. The total and free reducing sugars (TRS and FRS) were determined on sorghum juice extracted from each piece, after sucrose acid hydrolysis, by the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). The sucrose content was calculated by the difference between TRS and FRS multiplied by a conversion factor of 0.95 (ratio between molecular mass of sucrose and the sum of glucose plus fructose produced by the sucrose acid hydrolysis). The sugar yield (g m^{-2}) was defined as the product of total reducing sugars (g kg^{-1}) and stems dry biomass (g m^{-2}) of the four plants harvested in each experimental plot for sugar content estimation.

The dry biomass of sweet sorghum was also determined at harvest when the maximum value was achieved. The fresh sorghum biomass yield was determined by harvesting manually all sorghum plants in each separate line of each experimental plot. Stems and leaves were separated and then oven-dried at 70 °C to constant weight in order to gravimetrically estimate plant dry biomass. Total dry biomass was determined as the sum of stems and leaves dry biomasses. The dry weights of the four plants that had already been removed during the determination of the sugar yield were not considered in this analysis.

Relationships between applied factors and yield functions analysis

Sweet sorghum yield functions were determined in terms of biomass (total, stems, and leaves dry biomass), sugar content (reducing and non-reducing), and total sugar yields. Relationship of sweet sorghum yield with applied factors (nitrogen and sodium) during irrigation cycles was evaluated by stepwise multiple regression analysis as follows:

$$y = a_0 + a_1x_1 + a_2x_2 + \cdots + a_nx_n \quad (2)$$

where y is the sorghum yield to be predicted (biomass, sugar content, and sugar yield), $a_0, a_1, a_2, \ldots, a_n$ are the regression coefficients; and $x_1, x_2, \ldots, x_n$ are the applied factors, *i.e.*, nitrogen and sodium. In the process, two dummy variables (Y_1 and Y_2) were introduced as orthogonal polynomial coefficients to take into account the effect of each experimental year on crop yield. These two dummy variables considered the variations in ET_0 and in the total amount of the irrigation water applied (425, 522, and 546 mm in 2007, 2008, and 2009, respectively) along the crop seasons, and the existence of plagues, diseases, weeds, and all other external factors that might have influenced crop production during the three seasons. The orthogonal polynomial coefficients Y_1 assumed the values of -1, 0, and 1, while Y_2 assumed the values of 1, -2, and 1, when describing 2007, 2008, and 2009, respectively.

Means and coefficients of variation were calculated for the replicates from each treatment (three replicates for the biomass yield estimation corresponding to the three sorghum lines harvested in each experimental plot, and nine replicates for sugar yield estimation corresponding to the triplicates determined from the three samples composed of stems top, middle, and bottom sections from the plants harvested for sugar content estimation). For determining significant effects, mean values were compared using the least significant difference (LSD) test at $P = 0.05$ (Fisher, 1941).

RESULTS AND DISCUSSION

Mean total, stems and leaves dry biomass yield, mean total reducing sugars, sucrose content, and sugar yield of the individual plots and the coefficients of variation of the repetitions are presented in Table V. Table VI presents the yield functions obtained by stepwise multiple regression analysis for total dry biomass (Y_{total}), stems dry biomass (Y_{stems}), leaves dry biomass (Y_{leaves}), total reducing sugars content (Y_{TRS}), sucrose content (Y_{sucrose}) and sugar yield (Y_{sugar}).

Analysis of total dry biomass response curves

The models obtained for total (Y_{total}), stems (Y_{stems}), and leaves dry biomass (Y_{leaves}) explained 85.0% to 90.3% of the observed variations, with a total of 36 observations analysed (Table VI). Dry biomass responded significantly to the application of N and Na^+. Also, a significant interaction was found between these two input factors which affected yield response negatively. The response to one of the input factors was thus inversely dependent on the other factor. This inverse relation has been reported, for example, for maize (Pang and Letey, 1998; Shenker *et al.*, 2003) since the use of saline waters, without proper irrigation management, may lead to the accumulation of salts in the root zone which will increase the osmotic stress and reduce plant transpiration, N uptake, and eventually crop yield. In this experiment, sweet sorghum potential transpiration (T_{p}) varied between 360 and 457 mm in the different experimental plots and along the years. T_{p} values obtained in plots irrigated with saline waters (subgroup A) were generally lower than those obtained in plots irrigated with fresh waters (subgroup C) despite sweet sorghum, depending on the variety, is a mo-

TABLE V

Mean values of dry biomass (total, stems and leaves), total reducing sugars (TRS) content, sucrose content and sugar yield in sweet sorghum determined during the three seasons

Group	Subgroup	Dry biomass			TRS	Sucrose	Sugar yield
		Total	Leaves	Stems			
		$g\ m^{-2}$			$g\ kg^{-1}$		$g\ m^{-2}$
I	A	1 697.2bc[a] (0.22)[b]	643.5abc (0.24)	1 053.7cde (0.34)	273.8a (0.09)	175.4bc (0.20)	228.05bc (0.20)
	B	2 102.5ab (0.12)	738.8ab (0.31)	1 363.7abc (0.14)	265.1a (0.13)	177.0bc (0.29)	288.65ab (0.18)
	C	2 151.3ab (0.12)	810.8ab (0.20)	1 340.5abc (0.18)	256.1a (0.18)	160.4c (0.28)	293.18ab (0.29)
II	A	1 782.4bc (0.20)	670.1abc (0.34)	1 112.3f (0.17)	271.0a (0.13)	197.0abc (0.19)	242.36bc (0.16)
	B	2 159.8ab (0.15)	704.2abc (0.31)	1 455.6ab (0.11)	262.8a (0.23)	165.8c (0.39)	287.02ab (0.27)
	C	2 399.3a (0.13)	836.1a (0.29)	1 563.1a (0.12)	293.5a (0.09)	205.4abc (0.16)	340.44a (0.18)
III	A	1 572.8cd (0.09)	575.9bc (0.23)	996.9cdc (0.14)	276.2a (0.11)	193.8abc (0.20)	213.60bc (0.15)
	B	1 585.6cd (0.10)	476.8cd (0.31)	1 108.8bcd (0.11)	287.6a (0.06)	199.2abc (0.10)	248.58abc (0.15)
	C	1 949.4abc (0.16)	602.6abc (0.26)	1 346.7abc (0.22)	291.3a (0.16)	200.8abc (0.24)	299.62ab (0.28)
IV	A	1 035.3e (0.17)	329.1d (0.30)	706.2e (0.24)	317.4a (0.09)	240.6a (0.14)	166.91c (0.17)
	B	1 136.1de (0.29)	320.6d (0.54)	815.5de (0.22)	311.8a (0.05)	225.7ab (0.09)	180.69c (0.15)
	C	1 146.2de (0.16)	302.4d (0.31)	843.7de (0.18)	286.1a (0.14)	193.0abc (0.22)	166.90c (0.19)
LSD (0.05)		470.0	237.8	373.9	61.4	57.5	57.5

[a] Values within a column followed by the same letter(s) are not significantly different at $P < 0.05$ among subgroups.
[b] The values in brackets are the coefficients of variation obtained from the repetitions in each experimental plot.

TABLE VI

Yield functions and statistical indicators obtained for total dry biomass (Y_{total}), stems dry biomass (Y_{stems}), leaves dry biomass (Y_{leaves}), total reducing sugars content (Y_{TRS}), sucrose content ($Y_{sucrose}$) and sugar yield (Y_{sugar})

Item[a]	Y_{total}	Y_{stems}	Y_{leaves}	Y_{TRS}	$Y_{sucrose}$	Y_{sugar}
	$g\ m^{-2}$			$g\ kg^{-1}$		$g\ m^{-2}$
Independent variable						
Constant	1213.33***	844.47***	332.00***	302.22***	189.39***	189.48***
Y_1	- ns[b]	- ns	- ns	19.63***	- ns	34.64**
Y_2	-86.95**	-53.84*	- ns	-7.40**	-13.43***	- ns
N	53.84***	32.86***	21.50***	-7.83×10^{-1}***	- ns	6.65***
Na^+	-1.66×10^{-1}*	- ns	-1.72×10^{-1}**	- ns	3.14×10^{-2}***	- ns
N^2	-6.27×10^{-1}***	-3.84×10^{-1}***	-1.95×10^{-1}***	- ns	- ns	-7.65×10^{-2}***
$(Na^+)^2$	- ns	-5.24×10^{-5}*	1.26×10^{-4}***	- ns	- ns	-1.40×10^{-5}*
$N \times Na^+$	-5.27×10^{-3}*	-4.48×10^{-3}*	-3.72×10^{-3}***	- ns	-9.66×10^{-4}***	-9.53×10^{-4}*
$Y_1 \times N$	- ns	3.57**	- ns	- ns	8.28×10^{-1}***	1.22**
$Y_1 \times Na^+$	- ns	- ns	-7.38×10^{-2}**	- ns	- ns	- ns
$Y_2 \times N$	- ns	- ns	9.30×10^{-1}**	- ns	- ns	- ns
$Y_2 \times Na^+$	8.12×10^{-2}*	- ns	- ns	- ns	- ns	- ns
Statistical analysis						
n	36	36	36	36	36	36
SSR	6.68×10^6	2.74×10^6	1.33×10^6	2.15×10^4	3.94×10^4	1.59×10^5
SSE	7.19×10^5	4.84×10^5	1.19×10^5	1.49×10^4	1.46×10^4	3.11×10^4
R^2	0.903	0.850	0.917	0.592	0.730	0.836
F-ratio	44.91	27.35	44.44	15.47	20.94	24.73

*, **, ***Significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.
[a] Y_1 and Y_2 = orthogonal polynomial coefficients; N = nitrogen; Na^+ = sodium; N^2 = square of the nitrogen application; $(Na^+)^2$ = square of sodium application; $N \times Na^+$, $Y_1 \times N$, $Y_1 \times Na^+$, $Y_2 \times N$, and $Y_2 \times Na^+$ = product of one input factor (*e.g.*, nitrogen) by the other (*e.g.*, sodium); n = observations fitted; SSR = sum of squares due to regression; SSE = sum of squares due to error; R^2 = coefficient of determination.
[b] Not significant.

derately to highly tolerant crop to salinity (Maas, 1990). Nutrient uptake was also reduced due to the use of saline waters and the salinity build up in the soil profile as shown in Ramos *et al.* (2012). These authors quantified nitrogen uptake reductions of 18 kg ha^{-1} between plots in subgroups A and C using a state-of-the-art transient water dynamics and solute transport model.

Fig. 1 shows the response curves of total, stems, and leaves dry biomass yield to different levels of N

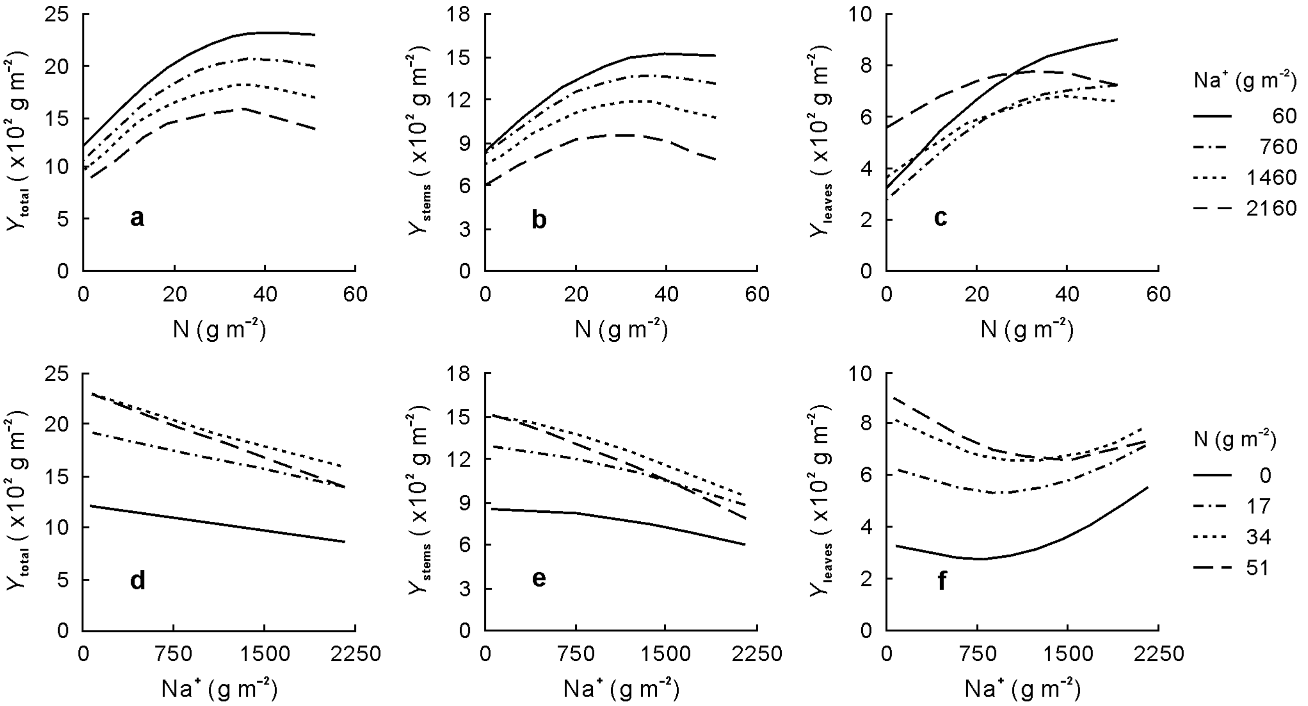

Fig. 1 Sweet sorghum total (Y_{total}; a, d), stems (Y_{stems}; b, e) and leaves (Y_{leaves}; c, f) dry biomass curves with decreasing returns to nitrogen (top), and to sodium (bottom). Mean annual yield response to one input factor (*e.g.*, nitrogen) was measured while maintaining all other input factors (*e.g.*, sodium) constant.

and Na^+. Response curves are presented for the mean annual yield. Regression analysis showed that total dry biomass response curves to N were quadratic with diminishing response for each incremental change of N. Each additional unit of N added less to the total output than the previous unit did. The partial derivates of the multiple regression equations obtained for Y_{total}, Y_{stems}, and Y_{leaves} (Table VI) with respect to N show the decrease in yield per unit increase in N levels. Increasing N application above the optimum level would not result in a direct increase of dry biomass. Also, as a result of the interaction found between N and Na^+, the optimum level of applied N decreased for each unit increase in Na^+ levels. The higher the amount of Na^+ applied the lower the level of N necessary to achieve maximum yield. For example, by equating the partial derivate to zero and solving for N, the maximum of total dry biomass was determined to be obtained for 42.7, 39.8, 36.8, and 33.9 g m^{-2} of N when considering increasing levels of 60, 760, 1 460, and 2 160 g m^{-2} of Na^+, respectively. The optimum levels of N obtained here were comparable with those obtained for maize grown in the same soil (Ramos *et al.*, 2009), even though sweet sorghum is considered to be more environmentally friendly than maize because of its relatively low N needs (Barbanti *et al.*, 2006; Almodares and Hadi, 2009). A similar decrease in N needs with increasing Na^+ levels was found while analyzing stems and leaves dry biomass separately.

If the experimental years are analysed individually, it is possible to observe that the optimum level of applied N for stems dry biomass, despite decreasing with increasing Na^+ levels as shown above, increased through the years (Y_1). Taking the level of 760 g m^{-2} of Na^+ as an example, maximum yield was achieved at optimum levels of 36.7, 38.3, and 42.9 g m^{-2} of N during the years of 2007, 2008, and 2009, respectively. This could most likely be explained with the total amount of irrigation water applied in each crop season. Higher amounts of water applied to the crop during 2008 (522 mm), and even higher in 2009 (546 mm), promoted salt leaching from the root zone, thus reducing the osmotic stress, increasing plant transpiration and allowing higher N uptake. The use of additional irrigation water to account for a leaching fraction and to counteract soil salinization/sodification is, in fact, one of the most common techniques presented in literature (US Salinity Laboratory Staff, 1954; Pang and Letey, 1998). Leaves dry biomass response to N application also varied along the years (Y_2).

Total dry biomass response to Na^+ was linear while stems and leaves dry biomass responses to Na^+ were quadratic (Fig. 1). The partial derivates of the multiple regression equations obtained for Y_{total}, Y_{stems}, and

Y_{leaves} (Table VI) with respect to Na^+ showed the decrease in yield per unit increase in Na^+ levels. Higher levels of Na^+ applied to the soil with the irrigation water led to total and stems dry biomass reduction. Identical results were reported in a vast number of experiments conducted for sweet sorghum under field or laboratory conditions (Netondo *et al.*, 2004; Begdullayeva *et al.*, 2007; Hassanein *et al.*, 2010; Vasilakoglou *et al.*, 2011). These authors found that dry biomass reductions with increasing soil salinity conditions may be attributed to reductions in the plant physiological parameters usually related to photosynthesis, namely reductions in the chlorophyll content, chlorophyll fluorescence and photosystem II quantum yield, and stomatal conductance. In Alvalade, as a result of the interaction obtained between N and Na^+, the decrease in yield per unit increase in Na^+ levels showed to be greater at higher levels of N. For the mean annual yield, sweet sorghum total dry biomass (Y_{total}) decreased by -0.17, -0.26, -0.35 and -0.43 g m^{-2} per unit increase of Na^+ applied to the soil, when the levels of N applied were considered to be 0, 17, 34, and 51 g m^{-2}, respectively. If the experimental years were analysed individually, similar tendencies were observed.

For stems dry biomass the rate of yield decrease per unit increase of Na^+ was only dependent of the N level applied (no interaction was found). Stems dry biomass response to Na^+ was more negative with higher N levels. Considering the level of 17 g m^{-2} of N as a first example, stems dry biomass decreased by 7%, 17%, and 32% when the levels of Na^+ applied were 760, 1 460, and 2 160 g m^{-2}, respectively. Considering now the level of 34 g m^{-2} of N, yield losses reached 10%, 22%, and 38% for the same levels of Na^+ applied.

Leaves dry biomass response to Na^+ was also dependent on the level of N applied to the crop. Leaves dry biomass decreased to each incremental change of Na^+, but only when considering the lower levels of this input factor. Higher levels of Na^+ apparently promoted leaves development. For the mean annual yield, the minimum of the quadratic function was found at Na^+ levels of 682, 933, 1 185, and 1 436 g m^{-2}, when assuming N levels of 0, 17, 34 and 51 g m^{-2}, respectively. Above those levels, leaves dry biomass increased at increasing levels of Na^+ due to the quadratic nature of the response curve to Na^+. However, as shown in Table V the effect of Na^+ was negative for both stems and leaves dry biomass. Therefore, the effect of Na^+ application was apparently more pronounced for stems dry biomass than for leaves.

Analysis of sugar yield response curves

The models obtained for TRS content (Y_{TRS}), sucrose content (Y_{sucrose}), and sugar yield (Y_{sugar}) explained 59.2% to 83.6% of the observed variations (Table VI). TRS content responded significantly to the application of N, but not to Na^+. Sucrose responded significantly to N and also to Na^+. However, N only affected significantly sucrose content while interacting with Na^+ and Y_1. Sugar yield response to the input factors was mostly in agreement with stems dry biomass responses since it was determined in part from this yield component. Sugar yield responded significantly to the application of N and Na^+. These two input factors also interacted negatively to affect sugar yield, in accordance with what was already described for stems dry biomass. The effect of N varied also in the response to each individual year and in the variability of mean annual yield. Fig. 2 shows the response curves of TRS content, sucrose content, and sugar yield to different levels of N and Na^+. Response curves are presented for the mean annual yield.

Regression analysis results showed that TRS response curves to N was linear. TRS content decreased at a constant rate of -0.783 g kg^{-1} per unit increase of N. A similar trend was reported by Wiedenfeld (1984) where stems carbohydrates content were reduced with increasing N application and uptake, despite increasing biomass yield. However, Smith and Buxton (1993) found little discernible effect on increasing fermentable sugar production with increasing N fertilization.

Sucrose content responded linearly to the application of N and Na^+. The partial derivate of the stepwise multiple regression equation obtained for Y_{sucrose} with respect to N showed that the effect of N on sucrose content depended on the level of Na^+ applied and the year in question. For the mean annual yield, sucrose content decreased at rates of -0.1, -0.7, -1.4, and -2.2 g kg^{-1} per unit increase of N, when considering the levels of 60, 760, 1 460, and 2 160 g m^{-2} of Na^+, respectively. In 2007, these rates were even higher with sucrose content responding more negatively to each incremental change of N. However, in 2009, some mixed tendencies were observed with sucrose content responding positively to N for levels of Na^+ up to 857 g m^{-2}, and negatively to N for levels of Na^+ above that quantity. This contrasting response is a result of the positive effect of Na^+ on sucrose content, as shown by the partial derivate of the same multiple regression equation with respect to Na^+. Sucrose content responded positively to Na^+ application but only for small levels of N, *i.e.*, for levels of N up to 32.5 g m^{-2}. For larger applications sucrose content response to each incremental change of Na^+ became negative. Thus, for levels of 0 and 17 g m^{-2} of N, the rate of sucrose increase per unit increase in Na^+ level was 0.031 and 0.015 g kg^{-1}, respectively.

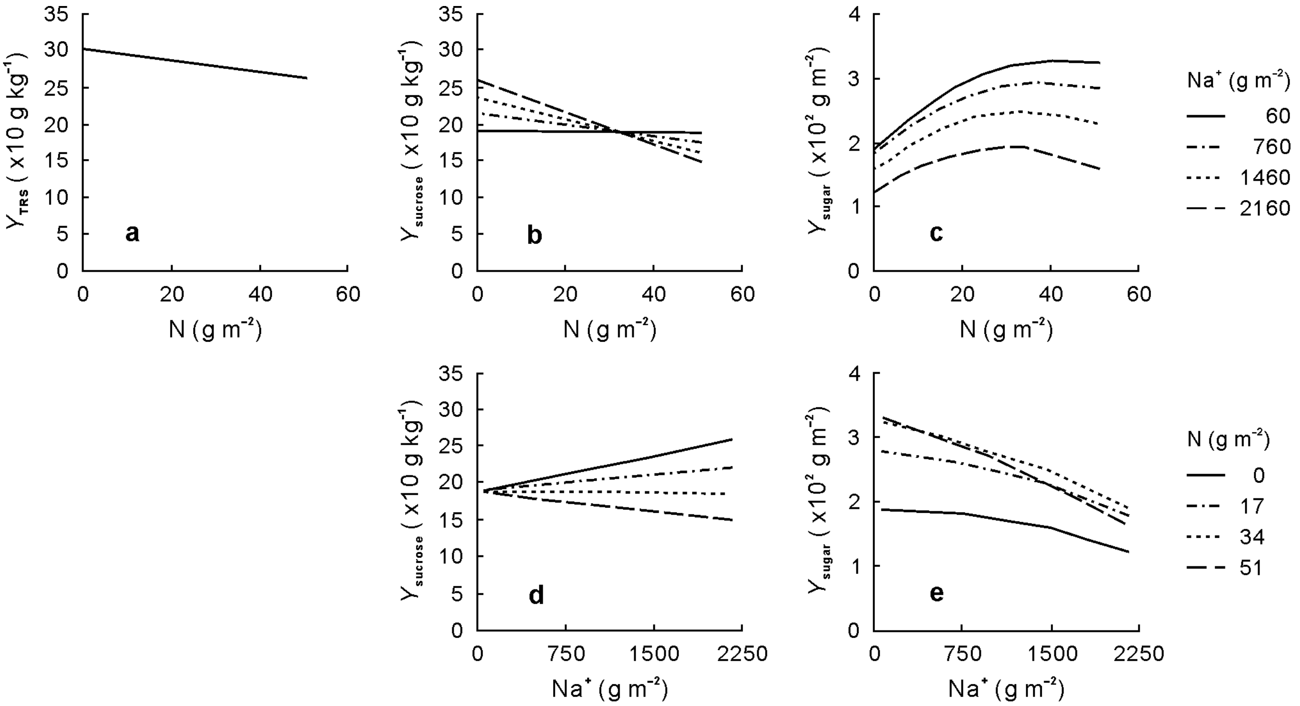

Fig. 2 Total reducing sugars content (Y_{TRS}; a), sucrose content (Y_{sucrose}; b, d), and sugar yield (Y_{sugar}; c, e) curves with decreasing returns to nitrogen (top), and to sodium (bottom). Mean annual yield response to one input factor (*e.g.*, nitrogen) was measured while maintaining all other input factors (*e.g.*, sodium) constant.

For levels of 34 and 51 g m^{-2} of N, sucrose content decreased at rates −0.001 and −0.018 g kg^{-1} per unit increase of the Na^+ level, respectively. This means that, in Alvalade sorghum plants affected by salinity and nitrogen stresses, *i.e.*, plants harvested from plots in groups III and IV, ended up having higher sucrose contents. Not many studies exist where the quality of sugar content has been related to soil salinity and to N fertilization. Therefore, comparing the results obtained here with others is not straightforward. Nevertheless, Almodares *et al.* (2007) found no effect of N fertilization on sucrose content. Also, Almodares *et al.* (2008a, b) found different responses for sucrose content in saline environments. As salinity increased, the sucrose content decreased in some varieties while for others sucrose content increased.

Sugar yield response curves to N and Na^+ were quadratic. The partial derivate of the multiple regression obtained for Y_{sugar} with respect to N yielded the same decrease in yield per unit increase in N levels reported in the dry biomass analysis. Increasing N application indefinitely would once more not result in a direct increase in sugar yield. The optimum level of applied N also decreased per unit increase of applied Na^+. For the mean annual yield, the maximum sugar yield was determined to be obtained for decreasing amounts of 43.1, 38.8, 34.4, and 30.0 g m^{-2} of N when considering increasing levels of 60, 760, 1 460, and 2 160 g m^{-2} of Na^+, respectively. The N needs were once more dependent on the year in question. Like for stems dry biomass and sucrose content, sugar yield and N needs progressively increased throughout the years. This is again likely explained by the increasing amounts of irrigation water applied per year and the increase of the leaching fraction available to remove Na^+ from the root zone, reducing the salinity stress and increasing plant transpiration.

The partial derivate of Y_{sugar} with respect to Na^+ showed that, for the same N level, the higher the amount of Na^+ applied, the greater the decrease in sugar yield would be. Taking the level of 60 g m^{-2} of N as a first example, sugar yield decreased by 4%, 16%, and 34% when the levels of Na^+ applied were 760, 1 460, and 2 160 g m^{-2}, respectively. If the level of 51 g m^{-2} of N is now considered, sugar yield losses would reach 13%, 30%, and 51% when applying the same levels of Na^+ as mentioned above. These results are in accordance with Almodares *et al.* (2008a, b). These authors reported while studying five sweet sorghum varieties that as salinity increased from 2 to 12 dS m^{-1}, total soluble carbohydrates decreased by 21%. Vasilakoglou *et al.* (2011) also reported that higher levels of soil salinity (6.9 dS m^{-1}) led to biomass reductions, which led to juice and total sugar yield reductions. They attributed these reductions to changes in sweet sorghum physiological parameters.

CONCLUSIONS

Sweet sorghum dry biomass yield showed to be dependent on the amount of N and Na^+ applied to each experimental plot. Sorghum dry biomass presented diminishing returns for each incremental change of N. Therefore, increasing N application above the optimum level did not result in an increase in total, stems or leaves dry biomass.

The use of saline irrigation waters also led to yield reduction. The higher the amount of Na^+ applied to the soil with the irrigation water, the lower the level of N necessary to achieve maximum yield. This effect was explained by an accumulation of salts in the root zone, which increased the osmotic stress and reduced plant transpiration, N uptake, and crop yield.

As for sugar content, total reducing sugars content and sucrose content were dependent on the amount of N applied to the crop. N had a negative effect on sugar content though. Sucrose content was also dependent on the amount of Na^+ applied, but in this case the salinity stress ended up promoting sucrose content.

Sugar yield embodied some of the trends observed mostly in sorghum dry biomass. Sugar yield presented similar diminishing returns for each incremental change of N. Higher levels of Na^+ applied to the soil also led to sugar yield reductions and minor N needs to achieve maximum sugar yield.

Stems dry biomass, sucrose content, and sugar yield progressively increased along the years of the experiment. This complementary effect between yield and years was attributed to the increasing amounts of irrigation applied throughout the years, and the increase of the leaching fraction which promoted salt leaching from the root zone, thus reducing the salinity stress and increasing plant transpiration, nitrogen uptake and sorghum yield.

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