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Sweet sorghum productivity for biofuels under increased soil salinity and reduced irrigation

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ABSTRACT

Sweet sorghum (*Sorghum bicolor* (L.) Moench.) is a drought-tolerant crop with high resistance to saline-alkaline soils, and sweet sorghum may serve as an alternative summer crop for biofuel production in areas where irrigation water is limited. A two-year study was conducted in Northern Greece to assess the productivity (biomass, juice, total sugar and theoretical ethanol yields) of four sweet sorghum cultivars (Sugar graze, M-81E, Urja and Topper-76-6), one grain sorghum cultivar (KN-300) and one grass sorghum cultivar (Susu) grown in intermediate (3.2 dS m^{-1}) or in high (6.9 dS m^{-1}) soil salinity with either low (120 mm) or intermediate (210 mm) irrigation water supply (supplemented with 142–261 mm of rainfall during growth). The soil salinity and irrigation water supply effects on the sorghum chlorophyll content index, photosystem II quantum yield, stomatal conductance and leaf K/Na ratio were also determined. The sorghum emergence averaged 75,083 plants ha^{-1} and 59,917 plants ha^{-1} in a soil salinity of 3.2 dS m^{-1} and 6.9 dS m^{-1}, respectively. The most affected cultivar, as averaged across the two soil salinity levels, was the Susu grass sorghum emerging at 53,250 plants ha^{-1}, followed by the Topper-76-6 sweet sorghum emerging at 61,250 plants ha^{-1}. The leaf K/Na ratio decreased with decreasing irrigation water supply, in most cases, but it was not significantly affected by soil salinity. The dry biomass, juice and total sugar yields of sorghum that received 210 mm of irrigation water was 49–88% greater than the yields of sorghum that received the 120 mm of irrigation water. Sorghum plants grown in a soil salinity of 3.2 dS m^{-1} produced 42–58% greater dry biomass, juice and total sugar yields than the yields of sorghum plants grown in a soil salinity of 6.9 dS m^{-1}. The greatest theoretical ethanol yield was produced by sweet sorghum plants grown in a soil salinity of 3.2 dS m^{-1} with 210 mm of irrigation water (6130 L ha^{-1}, as averaged across cultivar), and the Urja and Sugar graze cultivars produced the most ethanol (7620 L ha^{-1} and 6528 L ha^{-1}, respectively). Conclusively, sweet sorghum provided sufficient juice, total sugar and ethanol yields in fields with a soil salinity of 3.2 dS m^{-1}, even if the plants received 50–75% of the irrigation water typically applied to sorghum.

1. Introduction

Interest in sweet sorghum in Mediterranean environments is increasing because of the use of biofuels (from juice) and raw materials (from bagasse) for energy production (Barbanti et al., 2006). Sweet sorghum is characterized by high sugar content, mainly sucrose, fructose and glucose, in the juice of the stalks, from which ethanol can be easily produced and used as biofuel. For this reason, sweet sorghum has also become a popular energy plant throughout the world (Mastrorilli et al., 1999). Additionally, sweet sorghum biomass is used for fiber, paper, syrup and animal feed (Steduto et al., 1997).

Water is the principal limiting factor of crop production in many areas of the world. Salinity also causes great losses in agriculture by lowering yields of various crops. Land salinization is acute and widespread in Greece (Koukoulakis et al., 2000). In these areas, irrigation is needed to obtain maximal yield because decreasing the water supply by irrigation causes a significant reduction in seasonal evapotranspiration, aerial sorghum dry matter and grain yield (Berenguer and Faci, 2001).

Sweet sorghum grows in marginal areas because of its high tolerance to saline and drought conditions (Berenguer and Faci, 2001; Almodares and Hadi, 2009). Sweet sorghum has higher water-use efficiency than other summer crops under both well-watered and water-stressed conditions (Steduto et al., 1997). From the agronomic point of view, sweet sorghum is more environmentally friendly than maize because of its relatively low nitrogen needs (Barbanti et al., 2006) and water requirements (Mastrorilli et al.,

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1999). According to Almodares and Hadi (2009), sweet sorghum used for biofuel production may be an alternative crop to maize in marginal irrigated areas where the irrigation water is limited during crop development. Sweet sorghum has also been suggested as a good source for ethanol production because of its rapid growth rate, early maturity and high total energy value (Smith and Buxton, 1993). Moreover, sweet sorghum production is encouraged by new policies regarding nonfood crops in the European Union (Rexen, 1992).

Despite that the potential of sweet sorghum as an alternative energy crop has been emphasized (Smith and Buxton, 1993; Steduto et al., 1997), the ability of various sweet sorghum cultivars to grow under soil salinity and water deficiency field conditions has not been sufficiently determined. Screening sweet sorghum cultivars with the objective of meliorating saline soils are challenges to breeders, plant physiologists and agronomists. When considering the introduction of sweet sorghum cultivars, the acceptance of farmers and their willingness to integrate ecologically appropriate crops must be guaranteed. The motivation of farmers may increase if sorghum cultivars provide direct benefits, such as acceptable biomass and biofuel production, from land where other crops are unproductive (Almodares and Hadi, 2009).

In semiarid Mediterranean areas, the principal limiting factor of crop production is water applied as irrigation to maximize yields. Therefore, to achieve a better planning of available water resources and to establish irrigation strategies that optimize crop yield, it is necessary to know the crop response to a variable irrigation supply (Berenguer and Faci, 2001), especially in saline soils where water stress is increased (Ould Ahmed et al., 2008). The objective of this research was to assess the productivity of four sweet sorghum cultivars grown in intermediate (3.2 dS m^{-1}) or high (6.9 dS m^{-1}) soil salinity with either low (120 mm) or intermediate (210 mm) irrigation water supply under Mediterranean conditions. Sweet sorghum productivity was also compared with the productivity of one grain cultivar and one grass sorghum cultivar.

2. Materials and methods

2.1. Experimental site

A field experiment was conducted in 2007 (year 1) and was repeated in the same field in 2008 (year 2) at the Technological and Educational Institute Farm of Thessaloniki in Northern Greece (22°48′33″ E and 40°39′08″ N; elevation of 0 m). Experiments were carried out on a sandy loam (Typic Xeropsamment) soil with the following physicochemical characteristics; clay 56 g kg^{-1}, silt 180 g kg^{-1}, sand 764 g kg^{-1} and organic matter 9 g kg^{-1}. The soil had a pH value of 8.1 (1:2 H_2O). The mean monthly temperature and rainfall data recorded near the experimental area (over a distance of approximately 300 m) are shown in Fig. 1.

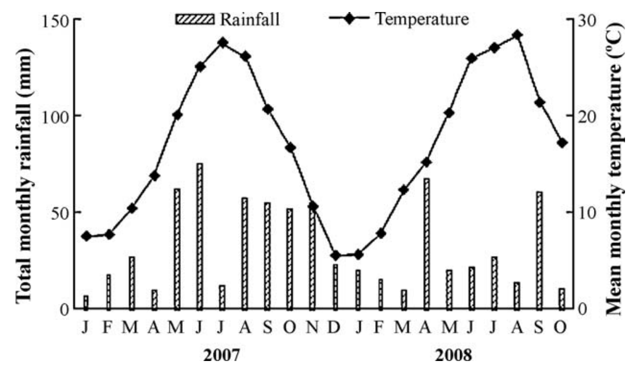

Fig. 1. Total monthly rainfall and mean monthly temperature during the experiment. The total rainfall received during May to September in year 1 and year 2 was 261 mm and 142 mm, respectively.

2.2. Treatments and experimental design

The experiment was established in a marginal field near the sea (over a distance of approximately 2.5 km) with great variability in soil salinity. In this field, two saline soil blocks (65 m by 20 m; 1300 m^2) were selected on the basis of their salinity level (part 1 with intermediate salinity and part 2 with high salinity according to Smith and Doran, 1996). The preplant soil analysis conducted in early May of both years showed that the initial soil salinity ranged from 2.5 dS m^{-1} to 3.6 dS m^{-1} (averaged 3.2 dS m^{-1} across samples) in part 1 and 5.4 dS m^{-1} to 7.3 dS m^{-1} (averaged 6.9 dS m^{-1} across samples) in part 2. The two salinity blocks (part 1 and part 2) were separated by a wide alley (50-m) to maintain the integrity of each salinity level. Moreover, the alley was imperative as long as the rainfall during the growing season may alternative salt concentration in soil. Each salinity block was divided into two irrigation sub-blocks (30 m by 20 m) separated by an alley (5 m). Furthermore, each irrigation sub-block was divided into four additional cultivar sub-blocks (four replicate sub-blocks; 14 m by 9 m). The four cultivar sub-blocks were separated by a wide alley (2 m). In each cultivar sub-block, six sorghum cultivars were planted in a randomized complete block design. The plot size was 6 m by 3 m, and each plot consisted of four sorghum rows.

Four sweet sorghum cultivars (cv. Sugar graze, Ypsilon, Thessaloniki, Greece; cv. M-81E, MAFES Foundation Seed Stocks, MS, USA; cv. Urja, Indian Agricultural Research Institute, New Delhi, India; and cv. Topper-76-6, MAFES Foundation Seed Stocks, MS, USA), one grass sorghum cultivar (cv. Susu, AlfaSeeds, Larissa, Greece) and one grain sorghum cultivar (cv. KN-300, Central Union of Seeds and Propagule, Thessaloniki, Greece) were planted by hand in rows (70 cm) to achieve an approximate density of 95,000 seeds ha^{-1}, which reflected the common practice in Greek sorghum fields. These sorghum cultivars have been recently introduced in Greece to be tested under Greek conditions. The planting dates were May 26, 2007 and May 21, 2008. Two days before sorghum planting, 130 kg N ha^{-1} as ammonium sulphate ($(NH_4)_2SO_4$), 50 kg P ha^{-1} as super phosphate ($Ca(H_2PO_4)_2$) and 65 kg K ha^{-1} as potassium sulphate (K_2SO_4) were broadcast applied and incorporated into the soil of all experimental plots. O-O-diethyl O-3,5,6-trichloro-2-pyridinyl phosphorothioate (Chlorpyriphos) was applied at a rate of 2 kg ha^{-1} at sorghum planting for insect management. After planting, all experimental areas were drip irrigated with 30 mm of water for sorghum emergence. The salinity of water used for irrigation was lower than 1.0 dS m^{-1} to avoid additional adverse effects, especially on sweet sorghum biomass. Drip irrigation was applied in rows that where 1.4 m apart and 0.5 m between the emitters, thus emitting 5.7 mm water h^{-1}. In each year, growth irrigations began in early July until harvest when the entire area was irrigated six times with a total volume of 90 mm (low water supply) or 180 mm (intermediate water supply) of water. During irrigation (approximately every 10 d), 15 mm (2.6 h for irrigation duration) or 30 mm (5.2 h for irrigation duration) of water was used in the 90 mm and 180 mm irrigation water supply, respectively. Before sorghum planting in both years, the soil water content was slightly lower than its field water capacity (approximately 0.18 m^3 m^{-3}). Furthermore, the total rainfall during the growing season was 261 mm in year 1 and 142 mm in year 2. The previous crop was two-row barley, which was harvested mid-June in 2006. Barley straw was baled and removed after harvest. Broadleaved weed control was achieved with 0.35 kg a.i. ha^{-1} of dicamba (3,6-dichloro-2-methoxybenzoic acid) (Banvel 48 SL, 480 g a.i. L^{-1}, BASF) while barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) was hand-removed. Other cultural practices were carried out according to the recommended production practices in Greek sorghum fields.

2.3. Measurements

The sorghum stand was counted at three weeks after planting (WAP) in the two central sorghum rows of each plot. The following physiological parameters were used to assess the reaction of the sorghum cultivars to soil salinity and irrigation water supply: (1) chlorophyll fluorescence and photosystem II quantum yield (Y); (2) chlorophyll content index (CCI); and (3) stomatal conductance (k_s), which is a factor related to leaf water potential. All measurements were conducted at two crucial sorghum growth stages as follows: (1) visible flag leaf at which rapid culm elongation occurs; and (2) half-bloom at which plant development has been completed (Vanderlip and Reeves, 1972). Measurements of the grass and grain sorghum cultivars were conducted approximately 10 d earlier that those of the sweet sorghum cultivars due to their earlier growth. Two measurements per plant were made on the upper leaves of five marked plants in the two center rows of each plot. Measurements of the chlorophyll fluorescence parameters were made on the upper (adaxial) surface of leaves using a chlorophyll fluorometer (MINI-PAM, Miniaturised Pulse-Amplitude-Modulated photosynthesis yield analyzer, Walz Co, Germany) with measurement light intensity of 0.15 μmol m^{-2} s^{-1}, frequency of 0.6 kHz and saturation pulse intensity of 16,000 μmol m^{-2} s^{-1} for 0.8 s to determine fluorescence at steady-state (F_s) and the maximal fluorescence after saturation flash ($F_{m'}$). The Y was calculated using the following equation: $Y = (F_{m'} - F_s)/F_{m'}$. The CCI was determined using a chlorophyll content meter (Opti-Scieces, model CCM-200, ADC Bio-Scientific Ltd., UK). The CCM-200 instrument uses calibrated light emitting diodes and receptors to calculate the CCI, which is defined as the ratio of transmission at 931 nm to 653 nm through a leaf sample and is strongly correlated with the chlorophyll concentration in leaves. Stomatal conductance was determined on the lower (abaxial) surface using a diffusion porometer (Leaf Porometer, model SC-1, Decagon Devices Inc., WA, USA). The upper leaves were selected for the measurements because of their higher stomatal conductance (lower resistance) (Turner and Begg, 1973). Conductance measurements were conducted on the abaxial leaf surface because Turner and Begg (1973) reported that the sorghum adaxial and abaxial epidermises have the same stomatal resistance. For the Y, CCI and k_s data, the average of ten measurements per plot was used for further data analyses.

At the half-bloom growth stage, ten leaves from the five marked plants of each plot were collected for determination of the K/Na ratio. An increase in leaf K/Na ratio has been suggested as a possible mechanism of sorghum tolerance to salt stress (Boursier and Läuchli, 1990). The leaf samples were dried at 65 °C for 72 h and were ground in a Wiley mill through a 1-mm screen. The K and Na content of the leaf samples were then determined by a photometry method using a frame photometer (Model Jenway PFP7, Spectronic Analytical Instruments, UK). The K/Na ratio was then calculated and used for further data analysis.

At harvest, the fresh sorghum biomass yield was determined by hand-harvesting the sorghum plants in the two central rows of each plot at the hard dough stage of each sorghum cultivar (Vanderlip and Reeves, 1972), which was approximately 35 d after blossom. According to Tsuchihashi and Goto (2004) and Zhao et al. (2009), sweet sorghum provides the greatest biomass and total sugar yield at this stage. In particular, the grain and grass cultivars were harvested at approximately 13 WAP, and the Sugar graze and Topper-76-6 cultivars were harvested at approximately 15 WAP while the M-81E and Urja cultivars were harvested at approximately 16 WAP. From each plot, ten randomly selected plants were chopped into 1-cm long pieces, and 4 kg of chopped biomass samples was used for determination of juice and dry biomass yields. The milling of sorghum chopped biomass was performed in a single step with a two roller machine miller without water imbibition. The sorghum juice was extracted from the milled biomass with a rotating hand press and was then weighed. The Brix degree of sorghum juice was measured with an automatic lab refractometer (Model ATR-ST, Topac, MA, USA). For dry matter determination, plant fractions (1 kg) were air-dried in the shade for 3 d and oven-dried at 65 °C for 24 h to a constant weight.

Table 1
Sorghum plant number as affected by soil salinity and cultivar main effects at three weeks after planting. Means are averaged across two years.

	Plants ha^{-1}	
Soil salinity (dS m^{-1})		
3.2	75,083	a[a]
6.9	59,917	b
Sorghum cultivar		
Sugar graze	76,750	a
M-81E	65,500	b
Urja	71,000	ab
Topper-76-6	61,250	bc
Susu	53,250	c
KN-300	77,250	a
CV, %	21.6	

[a] Means within each salinity or cultivar effect followed by the same letter are not significantly different according to the Fisher's protected LSD test at $P = 0.05$.

2.4. Calculations and statistical analyses

Total sugar content was calculated based on its correlation to Brix degree values using the following equation as estimated by Liu et al. (2008);Total sugar content(%) = [0.8111 × Brix(%)] − 0.3728

For the calculation of theoretical ethanol production from sweet sorghum fresh biomass, the equations reported by Sakellariou-Makrantonaki et al. (2007) and Zhao et al. (2009) were modified as follows: total ethanol yield (L ha^{-1}) = total sugar content (%) × fresh biomass (Mg ha^{-1}) × 6.5 (conversion factor of ethanol from sugar) × 0.85 (process efficiency of ethanol from sugar) × (1.00/0.79) (specific gravity of ethanol; g mL^{-1}).

All data were analyzed over year, soil salinity and irrigation water supply using a single factorial (sorghum cultivars) approach, which was replicated four times. The homogeneity of variances was examined with the Bartlett's test. Data for the leaf K/Na ratio and stomatal conductance of the six sorghum cultivars before the ANOVA were log(x)-transformed to reduce their heterogeneity. However, the means presented were back-transformed values. The MSTAT program (MSTAT-C, 1988) was used to conduct the analyses of variance. Fisher's protected LSD procedures were used to detect and separate mean treatment differences at $P = 0.05$.

3. Results

3.1. Sorghum emergence

Sorghum emergence at 3 WAP was affected by soil salinity ($P < 0.001$) and depended on the type of sorghum cultivar ($P < 0.001$). In particular, the sorghum plant density in a soil salinity of 6.9 dS m^{-1} was 20% lower (59,917 plants ha^{-1}) than the plant density in a soil salinity of 3.2 dS m^{-1} (75,083 plants ha^{-1}) (Table 1). Averaged across the two soil salinity levels, the Susu grass sorghum cultivar (53,250 plants ha^{-1}) was the most affected cultivar. The Sugar graze sweet sorghum, Urja sweet sorghum and KN-300 grain sorghum showed the greatest emergence (76,750 plants ha^{-1}, 71,000 plants ha^{-1} and 77,250 plants ha^{-1}, respectively). However, the emergence of the M-81E and Topper-76-6 sweet sorghums was similar to the emergence of the Urja sweet sorghum.

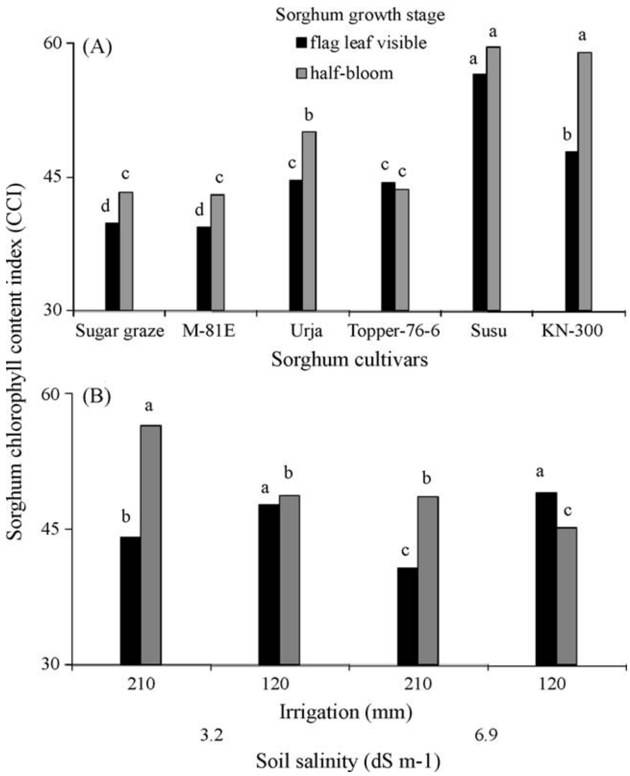

Fig. 2. The chlorophyll content index (CCI) at two growth stages of sorghum as affected by (A) cultivar and (B) interaction between soil salinity and irrigation water supply. The irrigation water during growth was supplemented with a total rainfall of 261 mm in year 1 and 142 mm in year 2. The means within each figure and sorghum growth stage followed by the same letter are not significantly different according to the Fisher's protected LSD test at $P = 0.05$.

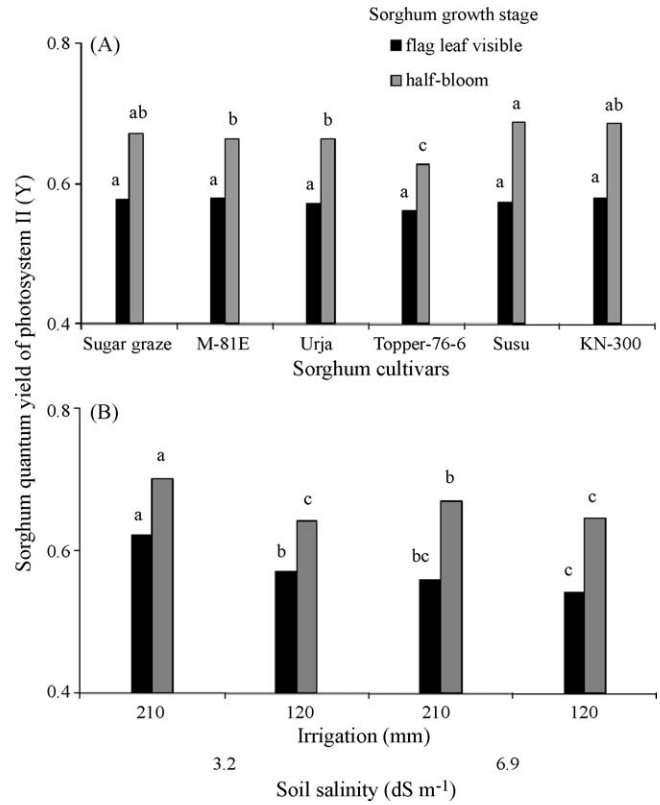

Fig. 3. The photosystem II quantum yield (Y) at two growth stages of sorghum as affected by (A) cultivar and (B) interaction between soil salinity and irrigation water supply. The irrigation water during growth was supplemented with a total rainfall of 261 mm in year 1 and 142 mm in year 2. The means within each figure and sorghum growth stage followed by the same letter are not significantly different according to the Fisher's protected LSD test at $P = 0.05$.

3.2. Physiological parameters

The sorghum chlorophyll content index (CCI) and photosystem II quantum yield (Y) at the visible flag leaf and half-bloom growth stages were affected in most cases by the year ($P < 0.001$), soil salinity ($P < 0.001$), irrigation water supply ($P < 0.001$), sorghum cultivar ($P < 0.001$) and interaction between soil salinity and irrigation water supply ($P < 0.05$). The means of the sorghum cultivar and means of the interaction between soil salinity and irrigation water supply are presented in Figs. 2 and 3.

Generally, the Susu grass and KN-300 grain sorghum cultivars had greater CCIs than the sweet sorghum cultivars (Fig. 2A). The increase of soil salinity decreased the sorghum CCI from 7% to 13% (Fig. 2B). At the visible flag leaf growth stage, the sorghum plants that received an irrigation water supply of 120 mm had greater CCIs than the plants that received an irrigation water supply of 210 mm. At the half-bloom growth stage, however, the sorghum plants that received an irrigation water supply of 120 mm had lower CCIs than the plants that received an irrigation water supply of 210 mm.

At the visible flag leaf growth stage, the Y did not differ among sorghum cultivars (Fig. 3A). At the half-bloom growth stage, however, the Sugar graze, Susu and K-300 cultivars had a greater Y than that of the Toper-76-6 cultivar. Generally, the decrease in irrigation water supply slightly decreased (3–8%) the Y of sorghum cultivars while the increase in soil salinity slightly decreased (2–7%) the Y of sorghum cultivars (Fig. 3B).

At both growth stages, the sorghum leaf stomatal conductance (k_s) was affected by the irrigation water supply ($P < 0.001$) and sorghum cultivar ($P < 0.001$). Averaged across cultivar, the leaf k_s was greater at the visible flag leaf growth stage (128 mmol $(m^2\,s)^{-1}$ and 160 mmol $(m^2\,s)^{-1}$ for cultivars that received an irrigation water supply of 120 mm and 210 mm, respectively) than at the half-bloom growth stage (82 mmol $(m^2\,s)^{-1}$ and 100 mmol $(m^2\,s)^{-1}$, respectively) (Table 2). At the visible flag leaf growth stage, the leaf k_s values of the M-81E, Urja and Topper-76-6 cultivars were greater

Table 2
Sorghum leaf stomatal conductance (k_s) as affected by irrigation water supply, soil salinity and cultivar main effects at the visible flag leaf and half-bloom growth stages. Means are averaged across two years.[a,b]

	Sorghum growth stage	
	Visible flag leaf Stomatal condutance (k_s) mmol $(m^2\,s)^{-1}$	Half-bloom
Irrigation water supply (mm)		
120	128 b[c]	82 b
210	160 a	100 a
Soil salinity (dS m^{-1})		
3.2	152 a	85 a
6.9	135 a	98 a
Sorghum cultivar		
Sugar graze	119 b	82 a b
M-81E	171 a	91 ab
Urja	171 a	95 ab
Topper-76-6	161 a	99 a
Susu	129 b	104 a
KN-300	119 b	77 b
CV, %	6.2	7.5

[a] Data before the analysis of variance were log(x)-transformed, but the mean values presented are back transformed.
[b] Irrigation water during growth was supplemented with a total rainfall of 261 mm in year 1 and 142 mm in year 2.
[c] Means within each main effect and growth stage followed by the same letter are not significantly different according to the Fisher's protected LSD test at $P = 0.05$.

Table 3
Significance levels for the leaf K/Na ratio and yield components of six sorghum cultivars grown in 2007 (year 1) and 2008 (year 2).[a]

Source	df	Significance of F ratio						
		K/Na	Fresh biomass	Dry weight	Juice	Total sugar	Brix degree	Theoretical ethanol
Year (Y)	1	***	***	***	ns	***	***	***
Soil salinity (SS)	1	ns[b]	***	***	***	***	ns	***
Y × SS	1	*	***	***	***	***	ns	***
Irrigation supply (IS)	1	***	***	***	***	***	ns	***
Y × IS	1	ns	***	***	***	***	ns	***
SS × IS	1	***	***	***	***	***	ns	***
Y × SS × IS	1	**	***	***	**	***	*	***
Replications (Y × SS × IS)	24	ns	***	***	***	***	***	***
Sorghum cultivar (SC)	5	***	***	***	***	***	***	***
Y × SC	5	***	***	***	***	*	***	***
SS × SC	5	***	***	***	***	***	*	***
Y × SS × SC	5	***	*	***	**	***	ns	***
IS × SC	5	***	***	***	***	***	***	***
Y × IS × SC	5	***	***	**	***	***	ns	***
SS × IS × SC	5	**	**	***	***	***	ns	***
Y × SS × IS × SC	5	**	ns	ns	ns	ns	ns	ns
Error	120							
CV, %		14.9	18.0	18.1	20.8	21.0	8.9	18.9

[a] Data for the leaf K/Na ratio of the six sorghum cultivars before the ANOVA were log(x)-transformed.
[b] ns, not significant.
* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.

(171 mmol (m^2 s)$^{-1}$, 171 mmol (m^2 s)$^{-1}$ and 161 mmol (m^2 s)$^{-1}$, respectively) than the values of the Sugar graze, Susu and KN-300 cultivars (119 mmol (m^2 s)$^{-1}$, 129 mmol (m^2 s)$^{-1}$ and 119 mmol (m^2 s)$^{-1}$, respectively). At the half-bloom growth stage, however, the leaf k_s did not differ among sweet sorghum cultivars.

3.3. Leaf K/Na ratio

The K/Na ratio in sorghum leaves was affected by the year, irrigation water supply, sorghum cultivar and interaction among the year, soil salinity, irrigation water supply and sorghum culti-

Table 4
Leaf K/Na ratio at the half-bloom growth stage of the six sorghum cultivars grown in two soil salinity conditions and that received two irrigation water supplies in 2007 (year 1) and in 2008 (year 2).[a,b]

Salinity	Irrigation	Cultivar	Year 1		Year 2	
dS m^{-1}	mm		K/Na ratio		K/Na ratio	
3.2	120	Sugar graze	8.1	efgh[c]	143.2	cde
		M-81E	5.8	h	36.4	hi
		Urja	5.6	h	97.9	efg
		Topper-76-6	7.7	fgh	60.3	fgh
		Susu	11.5	cdefg	15.2	jk
		KN-300	12.6	bcdefg	108.4	cdefg
	Irrigation effect		8.6		76.9	
	210	Sugar graze	22.6	abc	349.9	ab
		M-81E	19.3	abcd	549.5	a
		Urja	24.4	ab	222.3	bcd
		Topper-76-6	15.4	abcde	117.8	cdefg
		Susu	17.2	abcd	138.4	cdef
		KN-300	14.5	bcdef	249.5	abc
	Irrigation effect		18.9		271.2	
Salinity effect			13.7		174.1	
6.9	120	Sugar graze	7.0	gh	22.7	ij
		M-81E	16.0	abcd	70.8	efgh
		Urja	15.9	abcde	125.3	cdefg
		Topper-76-6	14.0	bcdef	125.0	cdefg
		Susu	6.6	gh	9.7	k
		KN-300	7.6	fgh	180.7	bcd
	Irrigation effect		11.2		89.0	
	210	Sugar graze	17.1	abcd	55.7	gh
		M-81E	28.4	a	126.5	cdefg
		Urja	21.2	abc	141.9	cde
		Topper-76-6	21.9	abc	188.8	bcd
		Susu	10.4	defgh	135.2	cdef
		KN-300	14.9	abcdef	405.5	ab
	Irrigation effect		19.0		175.6	
Salinity effect			15.1		132.3	
CV, %			18.7		12.8	

[a] Data before the analysis of variance were log(x)-transformed, but the mean values presented are back transformed.
[b] Irrigation water during growth was supplemented with total rainfall of 261 mm in year 1 and 142 mm in year 2.
[c] Means in each column followed by the same latter indicate no significant differences according to Fisher Protected LSD test at 5% level of significance.

Table 5

Fresh biomass yields, dry biomass yields, juice yields, Brix degree of juice, total sugar yield and theoretical ethanol yields of the six sorghum cultivars grown in two soil salinity conditions and that received two irrigation water supplies. Means are averaged across two years.[a]

Salinity (dS m^{-1})	Irrigation (mm)	Cultivar	Fresh biomass (Mg ha^{-1})		Dry biomass (Mg ha^{-1})		Juice (Mg ha^{-1})		Brix degree (%)		Total sugar (Mg ha^{-1})		Theoretical ethanol (L ha^{-1})	
3.2	120	Sugar graze	49.9	efg	16.1	efgh[b]	14.3	fgh	13.2	bc	1.47	fg	3591	de
		M-81E	61.2	de	23.7	cd	22.5	cd	10.2	hi	1.75	ef	3354	ef
		Urja	48.4	efg	15.8	efghi	13.9	fgh	12.1	cde	1.33	gh	3225	ef
		Topper-76-6	35.5	ghijkl	13.0	ghijk	14.0	fgh	11.1	fghi	1.15	hij	2087	hijk
		Susu	23.5	jkl	9.3	jkl	4.2	kl	11.2	efg	0.37	no	1433	lm
		KN-300	38.5	fghij	11.5	hijkl	7.5	ijkl	11.9	def	0.68	klm	2451	ghi
	Irrigation effect		42.8		14.9		12.7		11.6		1.13		2690	
	210	Sugar graze	88.6	ab	27.6	bc	28.3	bc	13.5	ab	2.98	b	6528	b
		M-81E	86.5	ab	31.0	ab	30.2	ab	11.5	def	2.71	bc	5447	c
		Urja	97.3	a	33.5	a	34.4	a	14.4	a	3.86	a	7620	a
		Topper-76-6	78.4	ab	27.1	bc	29.1	ab	11.4	ef	2.60	c	4926	c
		Susu	30.4	hijkl	10.9	hijkl	6.0	jkl	11.5	def	0.54	lmno	1913	ijkl
		KN-300	47.2	efgh	13.8	ghijk	10.1	hijk	10.2	ghi	0.80	kl	2615	gh
	Irrigation effect		71.4		24.0		23.0		12.1		2.25		4842	
Salinity effect			57.1		19.4		17.9		11.9		1.69		3766	
6.9	120	Sugar graze	40.8	fghi	12.6	hijkl	12.2	fghi	13.1	bc	1.25	ghi	2909	fg
		M-81E	43.2	fghi	16.4	efgh	12.3	fghi	10.1	i	0.95	jk	2375	ghij
		Urja	39.6	fghij	13.3	ghijkl	10.8	ghij	11.4	ef	0.95	jk	2454	ghi
		Topper-76-6	26.9	ijkl	11.7	hijkl	9.8	hijk	11.2	efgh	0.82	kl	1598	klm
		Susu	18.7	l	7.2	i	2.7	l	12.5	bcd	0.26	o	1264	m
		KN-300	29.7	ijkl	9.0	kl	5.3	jkl	11.8	def	0.47	mno	1850	jklm
	Irrigation effect		33.2		11.7		8.9		11.7		0.78		2075	
	210	Sugar graze	67.2	cd	20.5	def	20.5	de	13.2	bc	2.13	d	4870	c
		M-81E	60.4	de	20.9	de	20.9	de	10.6	i	1.78	e	3626	de
		Urja	54.6	def	18.5	defg	17.1	def	13.1	bc	1.79	e	3982	d
		Topper-76-6	47.8	efg	15.0	fghij	16.0	efg	11.7	def	1.51	efg	3246	ef
		Susu	19.9	kl	8.1	kl	2.9	l	11.7	def	0.27	o	1271	m
		KN-300	36.6	ghijk	10.3	ijkl	8.0	ijkl	10.2	ghi	0.64	lmn	2031	hijkl
	Irrigation effect		47.8		15.6		14.2		11.8		1.35		3171	
Salinity effect			40.5		13.6		11.5		11.7		1.07		2623	
CV, %			18.0		18.1		20.8		8.9		21.0		18.9	

[a] Irrigation water during growth was supplemented with a total rainfall of 261 mm in year 1 and 142 mm in year 2.

[b] Means in each column followed by the same latter indicate no significant differences according to Fisher Protected LSD test at 5% level of significance.

var (Table 3). Thus, data were analyzed separately for each year. The means of the interaction among the year, soil salinity, irrigation water supply and sorghum cultivar are shown in Table 4. Generally, the K/Na ratio in sorghum leaves was lower in year 1 than in year 2. Averaged across the year and soil salinity, the leaf K/Na ratio of sorghum plants that received an irrigation water supply of 210 mm was greater than the ratio of sorghum plants that received an irrigation water supply of 120 mm. In particular, the leaf K/Na ratio in the Sugar graze cultivar increased approximately 2.5 times with the increase in irrigation water supply. The increase in the K/Na ratio with increasing irrigation water supply was also the case for the M-81E and Urja cultivars when grown in a soil salinity of 3.2 dS m^{-1}. In most other cases, the increase in the irrigation water supply also resulted in a slight increase in the K/Na ratio of sorghum leaves. In most cases, however, the increase in soil salinity did not affect the K/Na ratio of sorghum leaves. Averaged across the year and irrigation water supply, the M-81E and Sugar graze cultivars had the greatest leaf K/Na ratio (152.8 and 131.0, respectively) in a soil salinity of 3.2 dS m^{-1}. The KN-300 cultivar had the greatest leaf K/Na ratio (152.2) in a soil salinity of 6.9 dS m^{-1}.

3.4. Sorghum yields

The fresh biomass, dry biomass, juice, total sugar and theoretical ethanol yields of the sorghum were affected by year (except for juice), soil salinity, irrigation water supply, sorghum cultivar and interaction among the soil salinity, irrigation water supply and sorghum cultivar (Table 3). The Brix degree value of sorghum was affected by the following parameters: year; sorghum cultivar; interaction between soil salinity and sorghum cultivar; and interaction between irrigation water supply and sorghum cultivar. As the ANOVA did not show significant interactions between the year and treatments the means of the interaction among soil salinity, irrigation water supply and sorghum cultivar are shown in Table 5.

Averaged across soil salinity and irrigation water supply, the fresh biomass yield of the Sugar graze, M-81E, Urja and Topper-76-6 sweet sorghum cultivars ranged from 40.8 Mg ha^{-1} to 88.6 Mg ha^{-1}, 43.2 Mg ha^{-1} to 86.5 Mg ha^{-1}, 39.6 Mg ha^{-1} to 97.3 Mg ha^{-1} and 26.9 Mg ha^{-1} to 78.4 Mg ha^{-1}, respectively (Table 5). The fresh biomass yield of the grass and grain sorghum cultivars ranged from 18.7 Mg ha^{-1} to 30.4 Mg ha^{-1} and 29.7 Mg ha^{-1} to 47.2 Mg ha^{-1}, respectively.

The dry biomass and juice yields of sorghum cultivars that received an irrigation water supply of 210 mm were 49% (19.8 Mg ha^{-1}) and 72% (18.6 Mg ha^{-1}), respectively, greater than the yields of sorghum cultivars that received an irrigation water supply of 120 mm (13.3 Mg ha^{-1} and 10.8 Mg ha^{-1}, respectively) (Table 5). Sorghum plants grown in a soil salinity of 3.2 dS m^{-1} produced 42% and 54% greater dry biomass (19.5 Mg ha^{-1}) and juice yields (17.9 Mg ha^{-1}), respectively, than the yields of sorghum plants grown in a soil salinity of 6.9 dS m^{-1} (13.7 Mg ha^{-1} and 11.6 Mg ha^{-1}, respectively). The greatest dry biomass yield was produced by sorghum plants grown in a soil salinity of 3.2 dS m^{-1} that received an irrigation water supply of 210 mm (24.0 Mg ha^{-1}, as averaged across the cultivar) with the Urja and M-81E cultivars as the most productive cultivars (33.5 Mg ha^{-1} and 31.0 Mg ha^{-1}, respectively). Under these conditions, the sorghum plants also produced the greatest juice yield (23.0 Mg ha^{-1}, as averaged across cultivar) with the Urja cultivar as the most productive cultivar (34.4 Mg ha^{-1}) followed by the M-81E (30.2 Mg ha^{-1}), Topper-76-6 (29.1 Mg ha^{-1}) and Sugar graze (28.3 Mg ha^{-1}) cultivars. Averaged across the cultivar, the lowest dry biomass and juice yields were produced by sorghum plants grown in a soil salinity of 6.9 dS m^{-1} and that received an irrigation water supply of 120 mm (11.7 Mg ha^{-1} and 8.9 Mg ha^{-1}, respectively). Under these conditions, the M-81E cultivar produced the highest dry biomass (16.4 Mg ha^{-1}) while the four sweet sorghum cultivars were the most productive regarding juice yield (9.8–12.3 Mg ha^{-1}). In a soil salinity of 6.9 dS m^{-1}, the M-81E, Sugar graze and Urja sweet sorghum cultivars that received an irrigation water supply of 210 mm had the greatest dry biomass yields (20.9 Mg ha^{-1}, 20.5 Mg ha^{-1} and 18.5 Mg ha^{-1}, respectively). The M-81E and Sugar graze cultivars also had the greatest juice yields (20.9 Mg ha^{-1} and 20.5 Mg ha^{-1}, respectively). Averaged across the soil salinity and irrigation water supply, the grass (Susu) and the grain (KN-300) sorghum cultivars had the lowest yields of dry biomass (8.9 Mg ha^{-1} and 11.2 Mg ha^{-1}, respectively) and juice (4.0 Mg ha^{-1} and 7.7 Mg ha^{-1}, respectively).

Averaged across the soil salinity and irrigation water supply levels, the Brix degree values of the Sugar graze, M-81E, Urja and Topper-76-6 sweet sorghum cultivars ranged from 13.1% to 13.5%, 10.1% to 11.5%, 11.4% to 14.4% and 11.1% to 11.7%, respectively. The Brix degree values of the grass and grain sorghum cultivars ranged from 11.2% to 12.5% and 10.2% to 11.9%, respectively (Table 5). The total sugar yield of sorghum cultivars that received an irrigation water supply of 210 mm was 88% greater than the yield of sorghum cultivars that received an irrigation water supply of 120 mm (1.80 Mg ha^{-1} and 0.96 Mg ha^{-1}, respectively). Sorghum plants grown in a soil salinity of 3.2 dS m^{-1} had a 58% greater total sugar yield than that of sorghum plants grown in a soil salinity of 6.9 dS m^{-1} (1.69 Mg ha^{-1} and 1.07 Mg ha^{-1}, respectively). The greatest total sugar yield was found in sorghum plants grown in a soil salinity of 3.2 dS m^{-1} that received an irrigation water supply of 210 mm (2.25 Mg ha^{-1}, as averaged across cultivar) with the Urja and Sugar graze cultivars as the most productive cultivars (3.86 Mg ha^{-1} and 2.98 Mg ha^{-1}, respectively). The lowest total sugar yield was produced by sorghum plants grown in a soil salinity of 6.9 dS m^{-1} that received an irrigation water supply of 120 mm (0.78 Mg ha^{-1}, as averaged across the cultivar) with the Sugar graze cultivar as the most productive cultivar (1.25 Mg ha^{-1}). The Sugar graze, Urja and M-81E sweet sorghum cultivars grown in a soil salinity of 6.9 dS m^{-1} that received an irrigation water supply of 210 mm had the greatest total sugar yield (2.13 Mg ha^{-1}, 1.79 Mg ha^{-1} and 1.78 Mg ha^{-1}, respectively). The grass (Susu) and grain (KN-300) sorghum cultivars had the lowest total sugar yield (0.36 Mg ha^{-1} and 0.65 Mg ha^{-1}, respectively).

The theoretical ethanol yield of sorghum cultivars that received an irrigation water supply of 210 mm was 68% greater than the yield of sorghum cultivars that received an irrigation water supply of 120 mm (4007 L ha^{-1} and 2383 L ha^{-1}, respectively, as averaged across the soil salinity and cultivar) (Table 5). Sorghum plants grown in a soil salinity of 3.2 dS m^{-1} had a 44% greater ethanol yield than the ethanol yield of sorghum plants grown in a soil salinity of 6.9 dS m^{-1} (3766 L ha^{-1} and 2623 L ha^{-1}, respectively). The greatest theoretical ethanol yield was produced by sorghum plants grown in a soil salinity of 3.2 dS m^{-1} that received an irrigation water supply of 210 mm (4842 L ha^{-1}, as averaged across the cultivar) with the Urja and Sugar graze cultivars as the most productive cultivars (7620 L ha^{-1} and 6528 L ha^{-1}, respectively). The lowest theoretical ethanol yield was produced by sorghum plants grown in a soil salinity of 6.9 dS m^{-1} that received an irrigation water supply of 120 mm (2075 L ha^{-1}, as averaged across the cultivar) with Sugar graze, Urja and M-81E the most productive cultivars (2909 L ha^{-1}, 2454 L ha^{-1} and 2375 L ha^{-1}, respectively). The Sugar graze and Urja sweet sorghum cultivars grown in a soil salinity of 6.9 dS m^{-1} that received an irrigation water supply of 210 mm had the greatest theoretical ethanol yield (4870 L ha^{-1} and 3982 L ha^{-1}, respectively) followed by the M-81E cultivar (3626 L ha^{-1}). The grass (Susu) and grain (KN-300) sorghum cultivars had the lowest theoretical ethanol yield (1470 L ha^{-1} and 2237 L ha^{-1}, respectively).

4. Discussion

According to the sorghum irrigation common practice in Greece, irrigation inputs range from approximately 520 mm to 660 mm (Sakellariou-Makrantonaki et al., 2007). Therefore, the low (90 mm + 30 mm) and intermediate (180 mm + 30 mm) irrigation water supplies applied during the current experiment further supplemented with rainfall water of 261 mm in year 1 and 142 mm in year 2 were approximately 50% and 75%, respectively, of the water amount typically applied in Greek sorghum fields.

The electrical conductivity of the two saline blocks selected for the current experiment ranged from 2.5 dS m^{-1} to 3.6 dS m^{-1} (averaged 3.2 dS m^{-1} across samples) and from 5.4 dS m^{-1} to 7.3 dS m^{-1} (averaged 6.9 dS m^{-1} across samples). These soil salinity conditions, which are typically present in Greece and other Mediterranean regions (Koukoulakis et al., 2000; Herrero and Perez-Coveta, 2005), exceed the threshold salinity levels of most crops and significantly reduce their productivity (Bresler et al., 1982).

The two irrigation levels were established after sorghum emergence. Therefore, irrigation did not affect this characteristic. The sorghum emergence in a soil salinity of 3.2 dS m^{-1} and 6.9 dS m^{-1} was 20% and 37% lower, respectively, than the emergence as predicted by the seed number planted. Similarly, Amzallag et al. (1990) found that the effect of 300 mol m^{-3} of NaCl during germination and emergence results in a reduced sorghum establishment. However, Berenguer and Faci (2001) studied the yield component of grain sorghum sown at four densities under variable water supply and reported that the different established plant densities ranging from 146,000 plants ha^{-1} to 300,000 plants ha^{-1} does not significantly affect sorghum aerial dry matter, grain yield and harvest index. They suggested that these results were due to a greater tiller production, greater number of grains per panicle and higher weight of grains that compensated for the smaller number of plants per m^2 in the lower plant densities.

In most cases, the increase in soil salinity caused a decrease in the sorghum CCI and *Y*. Under greenhouse conditions, Netondo et al. (2004a) found that the sorghum Chl a and Chl b are reduced from 58% to 70%, *Y* is reduced by 9% while the net CO_2 uptake is almost completely inhibited with increasing external NaCl concentration up to 200 mM. The sorghum CCI and *Y* values were also reduced with reducing irrigation water supply. Similarly, Singh and Singh (1995) found that the sorghum net photosynthesis significantly declines with increasing moisture stress.

An increase in soil salinity did not significantly affect the k_s of sorghum leaves. Under greenhouse conditions, however, Netondo et al. (2004b) found that the sorghum k_s value decreases with increasing external NaCl concentrations (up to 200 mM). In the current study, the k_s value was reduced when the irrigation water supply was reduced indicating stomatal closure with increasing leaf water deficit. Similarly, Turner et al. (1978) found that sorghum plants adjust osmotically in response to water deficits and that the osmotic potential at stomatal closure in the dried sorghum plants decreases with increasing leaf water deficit.

The sorghum leaf K/Na ratio was affected by the irrigation water supply, but it was not significantly affected by the soil salinity. Hoo et al. (1999) studied the relationship between the K/Na ratio and salt tolerance of eight grasses, and they found that the two *Sorghum* species studied were salt-tolerant because they could limit the uptake of Na. The greater K/Na ratio found in sorghum leaves in year 2 when compared to the ratio found in year 1 may be attributed to increased K accumulation in sorghum leaves during year 2 when compared to the K accumulation in year 1 (data not shown). This increased K accumulation in the sorghum leaves found in year 2 may be attributed to a greater K availability which may be in soil, compared with that in Year 1, maybe due to repeated K fertilization.

Sweet sorghum had a sufficient yield under combined increased soil salinity and reduced irrigation conditions. Based on this result, sweet sorghum may grow in semi-saline and semiarid areas where the irrigation water is limited. In particular, sweet sorghum plants grown in a soil salinity of 3.2 dS m^{-1} (characterized as intermediate salinity level) and that received an irrigation water supply of 210 mm had sufficient yields of dry biomass (27.1–33.5 Mg ha^{-1}), juice (29.1–34.4 Mg ha^{-1}), total sugar (2.60–3.86 Mg ha^{-1}) and theoretical ethanol (4926–7620 L ha^{-1}). However, these sorghum yields were enhanced by the initial soil water content and precipitation ranging from 141 mm to 262 mm during the growing season. The sorghum yields achieved in the current experiment were comparable to those traditionally found in Mediterranean areas. According to Mastrorilli et al. (1995), sweet sorghum produces up to 32 Mg ha^{-1} of biomass in Mediterranean conditions while Curt et al. (1995) found that the dry biomass of the Keller sweet sorghum cultivar in Spain ranges from 16.0 Mg ha^{-1} to 40.0 Mg ha^{-1}. Sakellariou-Makrantonaki et al. (2007) studied the water-use efficacy of the Keller cultivar grown in central Greece, and they found that the dry biomass and theoretical ethanol yields of the surface drip irrigated plants ranges from 21.0 Mg ha^{-1} to 33.6 Mg ha^{-1} and 5120 L ha^{-1} to 8390 L ha^{-1}, respectively. Moreover, in the dry lands of Indonesia, Tsuchihashi and Goto (2004) found that sorghum dry biomass and total sugar yields range from 39.5 Mg ha^{-1} to 47.9 Mg ha^{-1} and 2.61 Mg ha^{-1} to 2.86 Mg ha^{-1}, respectively.

The sorghum Brix degree value was not affected by soil salinity or irrigation water supply effects, which was in agreement with the results of Miller and Ottman (2010) who found that water stress does not affect sugar concentration in sweet sorghum. Under greenhouse conditions, however, Almodares et al. (2008) found that the total carbohydrates in sweet sorghum decreases by 21% as salinity increases from 2 dS m^{-1} to 12 dS m^{-1}.

The sorghum theoretical ethanol yield was reduced when the soil salinity increased or irrigation water supply decreased due a reduction in biomass, juice and total sugar yields. The reduction in sorghum yields found in increased soil salinity may be partially attributed to the reduction in the chlorophyll content and photosystem II quantum yield. Similarly, Begdullayeva et al. (2007) found that the sorghum dry biomass and grain yield decreases by 45% at high soil salinity (1.0–2.0% water-soluble salts) while Netondo et al. (2004a) found that increased external NaCl concentration during the sensitive vegetative phases reduces sorghum leaf growth and leaf area, thus, causing lower biomass production. However, reduced sorghum yields caused by a decrease in the irrigation water supply may be attributed to reduced chlorophyll content, reduced photosystem II quantum yield, stomatal closure and decreased sorghum leaf K/Na ratio. In particular, the reduction of irrigation water supply by 90 mm reduced the theoretical ethanol by 41%. This reduction may be explained by the increased salinity stress with increasing sorghum water stress due to increasing leaf water potential (Ould Ahmed et al., 2008). Similarly, Singh and Singh (1995) found a 54% reduction in sorghum dry biomass yield due to water deficit. According to Berenguer and Faci (2001), a decreasing water supply by irrigation produces a significant reduction in seasonal evapotranspiration, aerial sorghum dry matter yield and grain yield. Zhao et al. (2009) found that the M-81E cultivar grown in China with low soil salinity approximately 140 mm irrigation water supply and 554 mm of precipitation had a 51–109% greater ethanol yield (ranged from 8213 L ha^{-1} to 11,384 L ha^{-1}) than the ethanol yield of the M-81E cultivar grown in a soil salinity of 3.2 dS m^{-1} that received an irrigation water supply of 210 mm during the present study. Almodares and Hadi (2009) also reported that, the M-81E cultivar in Iran had a stem yield of 103.57 Mg ha^{-1}. The greater yield of the M-81E cultivar found in these experiments compared with the yield in our experiment may be explained by a greater total water supply (Farah et al., 1997).

5. Conclusion

The results of this study indicated that sweet sorghum provides sufficient yields even when grown under stresses of soil salinity and reduced irrigation. Sweet sorghum plants produce sufficient juice, total sugar and ethanol yields in fields with soil salinity up to 3.2 dS m^{-1} even though the plants receive 50–75% of the water regimes typically applied to sorghum. Therefore, sweet sorghum may be viable as an alternative crop system for bioethanol production under increased salinity and reduced irrigation conditions, especially in semi-saline and semiarid Mediterranean fields where the irrigation water is limited during crop development. Among the cultivars tested, the Sugar graze cultivar will provide sufficient bioethanol yield even in fields with a soil salinity of 6.9 S m^{-1}, presupposing an irrigation water supply of at least 75% of the water supply typically applied to sweet sorghum.

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