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Soil water effect on crop growth, leaf gas exchange, water and radiation use efficiency of *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel in semi-arid Mediterranean environment

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Abstract

Great effort has been placed to identify the most suited bioenergy crop under different environments and management practices, however, there is still need to find new genetic resources for constrained areas. For instance, South Mediterranean is strongly affected by prolonged drought, high vapour pressure deficit (VPD) and extremely high temperatures during summertime.

In the present work we investigated the soil water effect on crop growth and leaf gas exchange of *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel, a perennial, rhizomatous, herbaceous grass. Furthermore, the net increase of biomass production per unit light intercepted [radiation use efficiency (RUE)] and per unit water transpired [water use efficiency (WUE)] was also studied. To this end a field trial was carried out imposing three levels of soil water availability (I_{100}, I_{50} and I_0, corresponding to 100% of ETm restitution, 50% ETm and rainfed condition, respectively) under a semi-arid Mediterranean environment. Leaf area index (LAI), stem height, biomass dry matter yield, CO_2 assimilation rate, and transpiration rate resulted significantly affected by measurement time and irrigation treatment, with highest values in I_{100} and lowest in I_0. RUE was highest in I_{100} followed by I_{50} and by I_0; on the other hand, WUE was higher in I_0 than I_{50} and I_{100}. At LAI values higher than 2.0, 85% photosynthetically active radiation was intercepted by the *Saccharum* stand, irrespective of the irrigation treatment.

Saccharum spontaneum spp. *aegyptiacum* is a potential species for biomass production in environment characterized by drought stress, high temperatures and high VPD, as those of Southern Europe and similar semi-arid areas.

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Key words: *Saccharum spontaneum*; marginal land; leaf area index; biomass yield; radiation use efficiency; water use efficiency; CO2 assimilation rate.

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Introduction

Perennial, no-food grasses have been proposed as the most efficient species for biomass production due to their natural resource use efficiency, agronomic, environmental and social benefits (Cosentino *et al*., 2005, 2008; Zegada-Lizarazu *et al*., 2010). Recently, the Italian *Ministero dello Sviluppo Economico* (MISE) promoted the use of lignocellulosic, herbaceous species (*Panicum virgatum*, *Arundo donax*, *Miscanthus giganteus*), crop residues, dedicated forestry species and other no-food resources to reach the biofuel goal set in the RED (European Commission, 2009), with a compulsory consumption of these feedstock for second generation biofuels production starting from 2018 (1.2%, calculated on the basis of energy content *Gcal*) to progressively increase up to 2.0% in 2022 (Italian Regulation, 2014).

While *Miscanthus* spp., *Panicum virgatum* and *Arundo donax* have been proposed as the most suited species for cold, warm temperate and for Mediterranean environments of EU and US due to their ability to keep high and stable yields under variable environmental conditions and management practices (Cosentino *et al*., 2007, 2014; Zegada-Lizarazu *et al*., 2010; Strullu *et al*., 2011; Heaton *et al*., 2008; Arundale *et al*., 2014), there is still need to find new genetic resources for areas affected by severe drought, flood, salinity, pollution or other constraints. For instance, South Mediterranean is strongly affected by prolonged summer drought which in turn limits yields of several crops. Furthermore, high vapour pressure deficit (VPD) coupled with extremely high temperatures reduce leaf conductance, affecting CO_2 assimilation rate and thus yield (Kiniry *et al*., 1998; Flexas *et al*., 2007). In addition, climate change effects are supposed to increase both temperature and drought in the near future (Cosentino *et al*., 2012; IPCC, 2013).

Endemic species with drought resistant traits and able to maintain carbon assimilation during hot midday might enclose several advantages. Out of several perennial grasses widespread in semi-arid

Mediterranean, a plant from *Saccharum* genus (*Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel), perennial, rhizomatous, herbaceous, C4 photosynthetic pathway of *Poaceae* family shows those traits of biomass crop (Cosentino *et al*., 2015). Native from Northern Africa, *Saccharum spontaneum* ssp. *aegyptiacum* has distributed along the seacoasts of South-Eastern Sicily, Italy.

Generally, stress tolerance of a plant species is not only determined by the plant genes but also by morphological, phenological, physiological and biochemical traits (Grzesiak *et al*., 2013).

Photosynthetic capacity is the first process affected by drought in relation to stomatal closure that leads also to reduce water loss via transpiration (Flexas *et al*., 2007; Chaves *et al*., 2009). Changes in plant morphological components have been also reported, as for example a decrease in leaf area index, specific leaf area, plant height and biomass yield (Erice *et al*., 2010). Leaf area is the main determinant of the rate of intercepted photosynthetically active radiation (IPAR). Understanding factors controlling leaf area and the limitations due to stress my help to define productivity of a plant stand per unit land area under different environments (Kiniry *et al*., 1999; Dohleman and Long, 2009). Biomass productivity can be determined either by the net increase in plant dry matter per unit light intercepted [radiation use efficiency (RUE)], per unit water transpired [water use efficiency (WUE)] or per nutrient taken up, as for nitrogen [nitrogen use efficiency (NUE)] (Kiniry *et al*., 2011). In the present work we investigated the soil water effect on crop growth (*i.e*., stem height, leaf area index and dry biomass yield) and leaf gas exchange (*i.e*., net photosynthesis and transpiration rate) of *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel. Furthermore, the net increase of biomass production per unit light intercepted (RUE) and per unit water transpired (WUE) was also studied.

Materials and methods

Field trial set-up

Establishment was carried out in spring 2005 at the Experimental farm of Catania University, Italy (10 m a.s.l., 37°25' N lat., 15° 03' E long.) in a typical Xerofluvent soil (USDA, 1999 MISSING IN REF LIST).

Rhizomes of *Saccharum spontaneum* L. spp. *aegyptiacum* (Willd.) Hack. were collected in riparian areas of South-Eastern Sicily, Italy. Fresh rhizomes were split in pieces of approximately 100 g with 2-3 main buds and directly transplanted at a density of 1 rhizome m^{-2} in a previously prepared soil bed, which was ploughed in autumn, and then disk harrowed in early spring. A randomized block experimental design with three replications was applied, with a single plot measuring 15 m^2 (5x3 m). Before transplanting 100 kg N ha^{-1} and 100 kg P_2O_5 ha^{-1} as ammonium sulphate and supersphosfate, respectively, were supplied. Weeds were controlled manually during the year of establishment. No fertilization and weed control have been performed in the year onwards. Plantlets were kept in well-watered condition from the establishment to the end of summer time, subsequently the irrigation was suspended. Soil water availability was differentiated from the spring 2011, sixth growing season, by applying three levels of maximum evapotranspiration restitution (ETm): I_{100} (100% ETm), I_{50} (50% ETm) and I_0 (rainfed condition).

Irrigation was applied from the middle of May to the middle of September, namely during the period of maximum crop ET.

Irrigation system, water amount, water application and crop coefficients were as reported by Cosentino *et al*. (2015).

Measurements

Main meteorological parameters were measured by means of a weather station connected to a data logger (CR10; Campbell Scientific, Logan, UT, USA), located at a distance of 100 m by the experimental field. Gas exchange activities, as assimilation rate (A, mol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and leaf temperature (°C) were measured using a portable photosynthesis system (Li6400, Li-Cor Inc., Lincoln, NE, USA), at a flow rate of 500 mL min^{-1} and at ambient CO_2 concentration, during cloudless days and at time of maximum solar radiation (*e.g*., 12:00 to 2:00 pm). Measurements were scheduled from regrowth up to harvest time throughout the growing season (from March 2014 to February 2015) at approximately monthly intervals. VPD (kPa) was calculated at each date of gas exchange measurement, from minimum air humidity and maximum air temperature values recorded between 12:00 and 2:00 pm. Intrinsic WUE (iWUE) was calculated as the ratio between net photosynthesis and transpiration rate at each measurement time (mol CO_2 m^{-2} s^{-1}/ mmol H_2O m^{-2} s^{-1}), while crop WUE (cWUE) as the ratio between peak biomass production and the corresponding water used by the crop (CWU). The solar radiation (PAR) at the soil level, inside the stand and over the crop canopy, was recorded by means of a Line Quantum Sensor (Li-Cor Inc.). Thus, the fraction of PAR intercepted by the crop was calculated:

$$FPAR = \frac{PARin - PARs}{PARin} \tag{1}$$

where:
FPAR = fraction of PAR intercepted by the crop;
PARin = incident PAR (W m^{-2});
PARs = PAR at the soil level (W m^{-2}).

The relationship between periodic measurement of leaf area index (LAI) and the FPAR was described by an asymptotic equation [$y=1-e^{(-k*LAI)}$], where *k* represents the extinction coefficient. This formula was used to calculate the PAR intercepted by the crop, assuming a linear behaviour of LAI between subsequent sampling dates and daily PAR as 45% of the incident total solar radiation (Monteith, 1965; Meek *et al*., 1984; Kiniry *et al*., 1999):

$$IPAR = \sum_{i=1}^{n} PARi \text{ x } FPARi \tag{2}$$

where:
IPAR = cumulated intercepted PAR;
PARi = PAR at day *i* (calculated as 45% of total daily solar radiation);
FPARi = fraction of intercepted PAR at day *i*.

The relationships between the IPAR (MJ m^{-2}) and the corresponding aboveground yield (g DM m^{-2}) of the different treatments were calculated by means of linear regressions. RUE values were the slopes of the regressions of aboveground yield (g DM m^{-2}) as a function of cumulated IPAR (MJ m^{-2}). Crop RUE (cRUE) was also calculated as peak biomass production and the corresponding IPAR.

Dry biomass yield was determined from samples harvested after each measurement of gas exchange (at approximately monthly intervals). At each sampling, twelve randomly selected stems were taken from each treatment and replication, and the total tiller number in one square meter was also measured. To avoid any border effect, stems were cut from the centre of each plot. Thus dry biomass yield (Mg DM ha^{-1}) was obtained from the product of plant dry weight (leaves and stems) and plant density (no. m^{-2}) by 10,000 m^2.

Stem height was measured from the base of the cut up to last node (cm) and afterwards biomass was partitioned into stems and leaves. The former were oven dried at 105°C and kept until constant weight, the latter were used for LAI determination before to be dried as above.

Fresh green LAI was calculated as leaf area on ground area (m^2 m^{-2}) and measured by means of a Delta-T Area Measurement System (Delta-T Devices Ltd., Burwell, Cambridge, England). The area meter was calibrated against paper standards of known area. Green LAI was accepted when >50% green tissue was detected by visual score.

Statistical analysis

Stem height, LAI, DM yield, net photosynthesis, transpiration rate and iWUE were subjected to the GLM repeated measures ANOVA univariate approach, where *date* represents the within-factor and *irrigation* the between-factor (IBM SPSS Statistics 22). When the Mauchly's sphericity test failed to meet the assumption of sphericity, the univariate results were adjusted by using the Greenhouse-Geisser Epsilon and the Huynh-Feldt Epsilon correction factors. Following the univariate test satisfying the sphericity for within-subjects effects, the F-values and associated P-values for between-subjects effects were tested. It is important to point out that the tests of between-subjects effects are based on the average of the within-subjects effects. With a P-value less than 0.0001, statistical significance is accepted (using the criterion of 0.05). Crop WUE and cRUE were subjected to one-way analysis of variance (ANOVA) with *irrigation* as fixed factor. Differences between means were evaluated for significance using Bonferroni test. Effects were considered significant at P≤0.05. The Pearson product moment correlation coefficient at P≤0.05 was executed to measure the degree of linear relationships between the two variables, namely IPAR (MJ m^{-2}) and the aboveground yield (g DM m^{-2}).

Results

Meteorological trend

Annual rainfall was quite low during the whole growing season, reaching 414.4 mm from regrowth to final harvest. During crop maximum assimilation rates (February to October in the present environment) cumulated rainfall was only 180.2 mm, while the remaining events were registered between November 2014 and February 2015, 234.2 mm. Lower minimum temperatures were observed in December 2014 and January 2015 (about 1.0-2.0°C) as compared with the previous winter (about 3.0-4.0°C in February 2014). Maximum temperatures progressively increased to reach the highest values in July and August 2014 (30.9-32.3°C). However, September, October and November 2014 still maintained maximum temperatures at 30°C, 25°C and 21°C respectively. The solar radiation at the soil level was lowest in February (12.8 MJ m^{-2} d^{-1}, as monthly averaged) and highest in June-July (26.1-26.8 MJ m^{-2} d^{-1}, as monthly averaged). VPD greatly changed during the growing season, increasing from March 2014 (1.49 kPa), peaking on June, July and August (3.47, 3.55 and 3.38 kPa, respectively) to reach the lowest values on January and February 2015 (0.88 and 0.81 kPa, respectively), as shown in Figure 1.

Morpho-biometric traits, biomass yield, crop water use efficiency and crop radiation use efficiency

Stem height progressively increased from regrowth, reaching a plateau in November 2014 in all treatments. I_{100} showed the highest values (218.2 cm), while I_0 the lowest (127.5 cm). I_{50} was significantly different between both treatments, 206.4 cm (Figure 2). Mean difference between I_{100} and I_0 was 62.2 cm, between I_{100} and I_{50} was 20.4 cm and between I_{50} and I_0 was 41.8 cm. Leaf area index peaked at September 2014 (6.18, 4.75 and 2.58 in I_{100}, I_{50} and I_0, respectively) and subsequently declined down to 2.49 (I_{100}), 1.70 (I_{50}) and 1.25 (I_0) at harvest time (February 2015), as shown in Figure 3. Mean difference between I_{100} and I_0 was 2.06, between I_{100} and I_{50} was 1.04 and between I_{50} and I_0 was

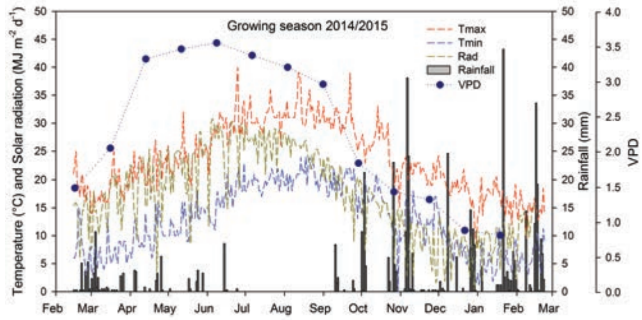

Figure 1. Meteorological trend during 2014/2015 growing season at the Experimental farm of Catania University, Italy (10 m a.s.l., 37°25' N lat., 15° 03' E long.). VPD, vapour pressure deficit.

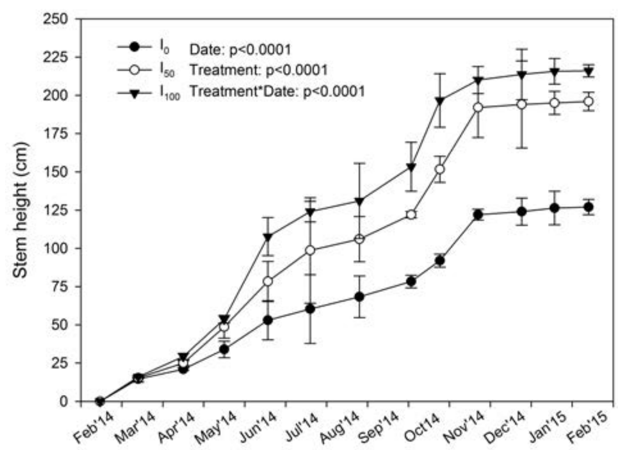

Figure 2. Stem height (cm) of *Saccharum spontaneum* L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA.

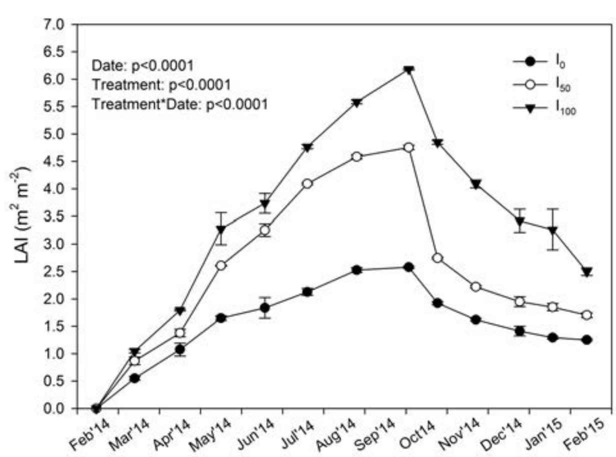

Figure 3. Leaf area Index (LAI) of *Saccharum spontaneum* L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA.

1.01. Biomass DM yield increased as described for stem height, peaking in November 2014. At this point maximum values were 17.4 Mg DM ha^{-1} in I_0, 24.4 Mg DM ha^{-1} in I_{50} and 34.3 Mg DM ha^{-1} in I_{100}. At harvest, the DM yield slightly decreased up to 14.1, 20.8 and 28.0 Mg ha^{-1} in I_0, I_{50} and I_{100}, respectively (Figure 4). Mean difference between I_{100} and I_0 was 8.8 Mg DM ha^{-1}, between I_{100} and I_{50} was 4.6 Mg DM ha^{-1} and between I_{50} and I_0 was 4.1 Mg DM ha^{-1}.

The effects of irrigation and date, as well as the interaction date*irrigation were highly significant ($P<0.0001$) on stem height, LAI and DM yield.

Crop WUE and cRUE are shown in Table 1. CWU was highest in I_{100}, intermediate in I_{50} and lowest in I_0. As result, the biomass yield as function of CWU led to significantly higher cWUE in I_0 than I_{50} and I_{100} (6.55 *vs* 5.29 and 5.19 g L^{-1}). On the other hand, the cRUE was significantly highest in I_{100} (1.29 g MJ^{-1}), intermediate in I_{50} (0.96 g MJ^{-1}) and lowest in I_0 (0.71 g MJ^{-1}).

Crop physiology and intercepted photosynthetically active radiation

CO_2 assimilation rate was similar before treatment differentiation (March-April 2014), afterwards it was highest in I_{100}, followed by I_{50} with a peak on May 2014 (32.4 and 26.8 mol CO_2 m^{-2} s^{-1} in I_{100} and I_{50}, respectively) (Figure 5). As the growing season approached summertime, and so temperatures and VPD increased, the assimilation rate of the crop decreased. Indeed, by looking at Figure 6A, air temperature was higher than 30°C in the interval May to September 2014. Leaf temperature increased as well, however a different trend was observed between treatments: both watered treatments (I_{100} and I_{50}) maintained lower leaf than maximum air temperature, while I_0 showed higher leaf than maximum air temperature on June, July and August. Hence, cumulated difference between leaf and maximum air temperature (Figure 6B) was positive in I_0 from June to August (0.21°C to 3.15°C), while it was always negative in both I_{50} and I_{100}.

The CO_2 assimilation rate became similar between treatments from November 2014 onward. Mean difference between treatments was 7.2 mol CO_2 m^{-2} s^{-1} in I_{100} and I_0, 3.4 mol CO_2 m^{-2} s^{-1} between I_{100} and I_{50} and 3.3 mol CO_2 m^{-2} s^{-1} between I_{50} and I_0.

Transpiration rate followed the same trend described for CO_2 assimilation rate. Maximum transpiration rate in watered treatments was measured on May 2014 (5.5 and 4.8 mmol H_2O m^{-2} s^{-1} in I_{100} and I_{50}, respectively) and subsequently declined to match with the rainfed condition (I_0) from December 2014 (Figure 7). Mean difference was 1.3 mmol H_2O m^{-2} s^{-1} between I_{100} and I_0, 0.5 mmol H_2O m^{-2} s^{-1} between I_{100} and I_{50}, 0.7 mmol H_2O m^{-2} s^{-1} between I_{50} and I_0.

The effect of irrigation and date, as well as the interaction date*irrigation was highly significant ($P<0.0001$) on both net photosynthesis and transpiration rate.

An increasing trend was shown by the instantaneous WUE (iWUE), however, only date effect was significant (Figure 8). Averaging measurements time, 6.80 mol CO_2/mmol H_2O were found in I_0, 6.34 mol

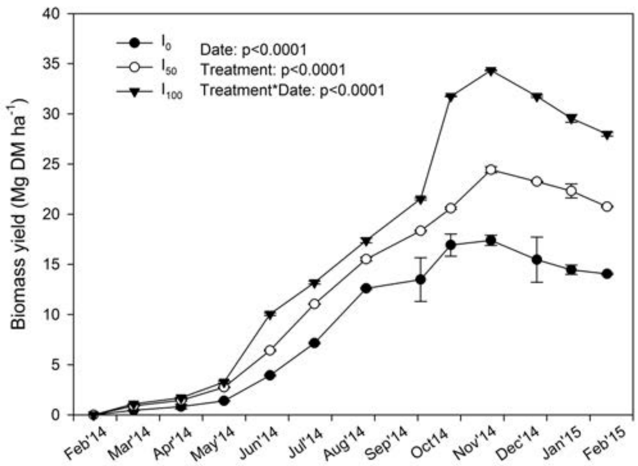

Figure 4. Aboveground biomass dry matter yield (Mg DM ha^{-1}) of *Saccharum spontaneum* L. ssp. aegyptiacum under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA.

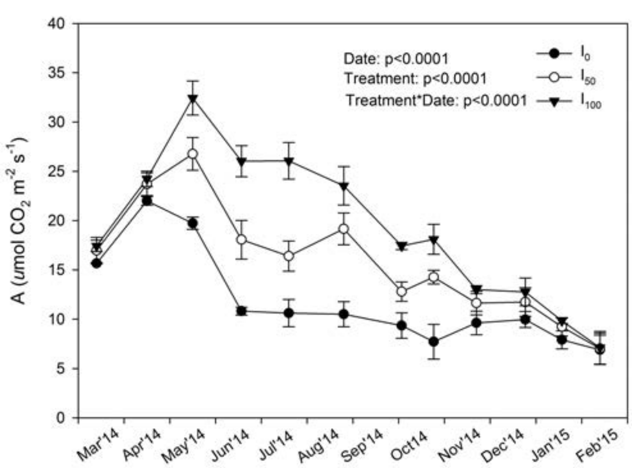

Figure 5. CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1}) of *Saccharum* spontaneum L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA.

Table 1. Crop water use (CWU), crop water use efficiency (cWUE), cumulated intercepted photosynthetically active radiation (IPAR) and crop radiation use efficiency (cRUE) of *Saccharum spontaneum* L. ssp. *aegyptiacum* (Willd.) Hackel.

Treatment	CWU (mm)	cWUE (g L^{-1})	IPAR (MJ m^{-2})	cRUE (g MJ^{-1})
I0	261.6	6.55[a]	2442.6	0.71[c]
I50	461.6	5.29[b]	2550.3	0.96[b]
I100	661.6	5.19[b]	2650.1	1.29[a]

[a–c]Different letters in the same column mean statistical significance according to Bonferroni test at P≤0.05.

CO_2/mmol H_20 in I_{50} and 6.45 mol CO_2/mmol H_20 in I_{100}.

FPAR approached the asymptote as the LAI was greater than 2.0. However, at these LAI values more than 85% PAR was intercepted by the *Saccharum* stand, irrespective of the irrigation treatment. I_0 intercepted 90% PAR due to a maximum LAI of 2.58, while both I_{50} and I_{100} were able to intercept all the available PAR (100%) due to a greater LAI.

A different light extinction coefficient (k) was found between treatments: 0.89 in I_0, 0.87 in I_{50} and 0.85 in I_{100} (Figure 9).

The net increase of biomass production (g m^{-2}) per unit light intercepted (MJ m^{-2}) led to highest RUE in I_{100} (1.26 g MJ^{-1}), followed by I_{50} (0.93 g MJ^{-1}) and I_0 (0.70 g MJ^{-1}), as shown in Figure 10.

According to the Pearson's test, high and positive correlation coefficients ($P \leq 0.0001$) were found in all relationships between the IPAR and the aboveground dry matter yield (0.97 in I_{100} and 0.98 in both I_{50} and I_0).

Discussion

It has been previously shown that *Saccharum spontaneum* spp. *aegyptiacum* possesses a range of agronomically desirable traits of biomass crop, as C_4 plant, high biomass yield, active assimilation rates during drought-stress periods, able to use water efficiently (Cosentino *et al*., 2015) and qualitative traits, as for second generation bioethanol production (Scordia *et al*., 2010, 2014).

Present results confirmed the ability of this crop to thrive on environments characterized by severe drought stress, high temperatures and high VPD during summer time.

CO_2 assimilation rate increased as the temperatures were favourable for growth (March-May), then a decreasing trend was observed throughout the growing season. Although the fully irrigation treatment did not experience water stress, the high temperatures and VPD during summertime, this latter increasing water loss from epidermal and guard cells (Mott and Parkhust, 1991), led to stomatal closure preventing dehydration of the crop but leading also to reduced carbon assimilation rates (Flexas *et al*., 2007).

It is worth to note that gas exchange between plant and atmosphere was still maintained even in the colder months of the growing season. This might be explained since the crop was able to conserve green LAI up to harvest time (1.25, 1.70 and 2.49, I_0, I_{50} and I_{100}, respectively).

Longer LAI maintenance allows intercepting more radiation, crop carbon assimilation and conversion into biomass throughout the growing season (Dolehman and Long, 2009).

However, we actually do not know if the carbon uptake in the coldest months was used by the crop to build up aerial biomass or if it served as carbon stock in the belowground for subsequent growing seasons. In this regard, further studies are needed to deal with this subject.

The biomass yield was comparable to that of other energy crops, as the C3 *Arundo donax* or the C4 *Miscanthus* x *giganteus* grown in the same experimental area. Cosentino *et al*. (2015) have shown that *Saccharum* yields are well related to CWU, with aboveground biomass as high as 37 Mg DM ha^{-1} when the crop used 1150 mm of water (rainfall and irrigation). The reduction of biomass yield from November to harvest is in accordance with the behaviour of other perennial, herbaceous, rhizomatous grasses, owed by leaf senescence and losses, as well as by nutrient translocation from above to belowground part (Heaton *et al*., 2004, 2008; Cosentino *et al*., 2007, 2014; Dohleman and Long 2009; Angelini *et al*., 2009; Nassi o di Nasso *et al*., 2011; Strullu *et al*., 2011). Water use was as efficient as that of sorghum (Cosentino, 1996), but higher than those of *Miscanthus* x *giganteus* (Cosentino *et al*., 2007) and *Arundo donax* (Cosentino *et al*., 2014) grown in semi-arid environment. Indeed, 5.19 g of biomass were produced with 1 liter of water in *Saccharum* I_{100} and 6.5 g in *Saccharum* I_0. *Miscanthus* and

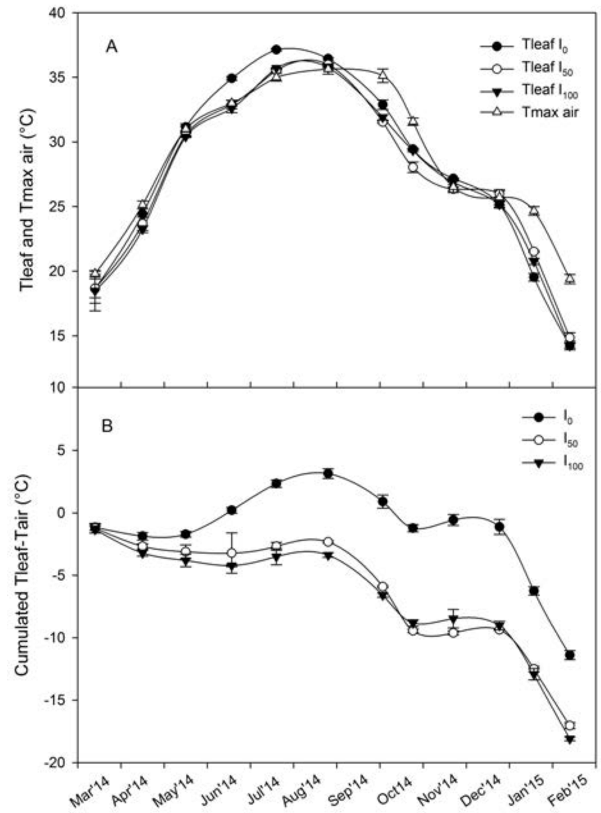

Figure 6. A) Leaf and maximum air temperature (°C) and B) cumulated difference between leaf and maximum air temperature (°C) of *Saccharum spontaneum* L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time.

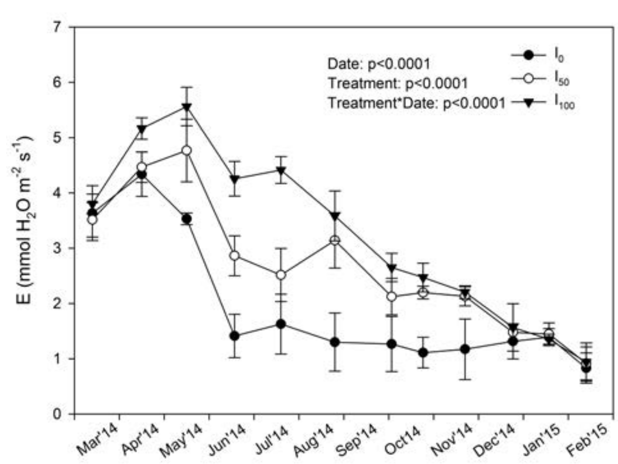

Figure 7. Transpiration rate (mmol H_2O m^{-2} s^{-1}) of *Saccharum spontaneum* L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA.

Arundo reached 4.83 g L^{-1} and 4.51 g L^{-1} with similar water supplied (Cosentino *et al*., 2007, 2014).

Slightly higher values were found with iWUE than cWUE in I_{50} and I_{100} treatments, while in rainfed condition (I_0) the values matched well between the two calculation methods (6.80 mol CO_2/mmol H_20 and 6.55 g L^{-1}, respectively). Both RUE and cRUE were highest in the fully irrigated treatment, followed by the intermediate and by the rainfed condition. The RUE calculated in both ways were very similar in all treatments. Our RUE (0.70-1.26 g MJ^{-1} for RUE and 0.71-1.29 g MJ^{-1} for cRUE) were higher than those of Cosentino *et al*. (2007), who reported 1.05 g MJ^{-1} with fully irrigated *Miscanthus* x *giganteus* and 0.56 g MJ^{-1} in rainfed conditions. Kiniry *et al*. (1999), on the other hand, showed RUE values of 1.6-5.0 g MJ^{-1} with switchgrass (*Panicum virgatum*), 0.5-1.8 g MJ^{-1} with sideoats grama (*Bouteloua curtipendula*), 1.0-1.9 g MJ^{-1} with big bluestem (*Andropogon gerardii*) and 1.9-2.6 g MJ^{-1} with eastern gamagrass (*Tripsacum dactyloides*) grown in Texas, USA.

In irrigated and non-irrigated *Miscanthus* x *giganteus* grown in Texas, Kiniry *et al*. (2011) found a RUE of 1.14-2.39 g MJ^{-1} and 0.48-1.42 g MJ^{-1}, respectively. Although biomass DM yield of *Saccharum* was substantial, RUE might resemble low values according to what found in literature with C4 crops. However, it is worth to note that RUE is strongly affected by VPD, in a similar fashion as it does for CO_2 assimilation rate (Kiniry *et al*., 1998). For instance, Bunce (1982) found that maize CO_2 assimilation rate at VPD of 2.5 kPa was 85% of that at VPD of 1.0 kPa, which corresponded to a 25% RUE reduction (Stockle and Kiniry, 1990). El-Sharkawy *et al*. (1985) reported that maize and sorghum CO_2 assimilation rate at VPD of 4.0 kPa were 59% and 70% of that at VPD of 1.25 kPa; such relative RUE would be 48% for maize and 74% for sorghum (Stockle and Kiniry, 1990).

Furthermore, Kiniry *et al*. (1998) have shown a liner decrease of RUE as function of VPD, highlighting that VPD during the light period (as measured in this work) is higher than the VPD averaged over 24 h period and have a greater impact on both CO_2 assimilation rate and RUE. Therefore, as also argued by Foti *et al*. (2003) and later by Cosentino *et al*. (2007), VPD in Mediterranean semi-arid environment is very high (often reaching 4.0 kPa) and might introduce downward bias in RUE calculation. It would be wise to focus on areas with similar environmental conditions in order to compare physiological parameters of a given crop category.

Conclusions

Saccharum spontaneum spp. *aegyptiacum* is a potential species for biomass production in environment characterized by drought stress, high temperatures and high VPD, as those of southern Europe and similar semi-arid areas.

The long green LAI maintenance and CO_2 assimilation, the high net increase of biomass production per unit light intercepted (RUE) and

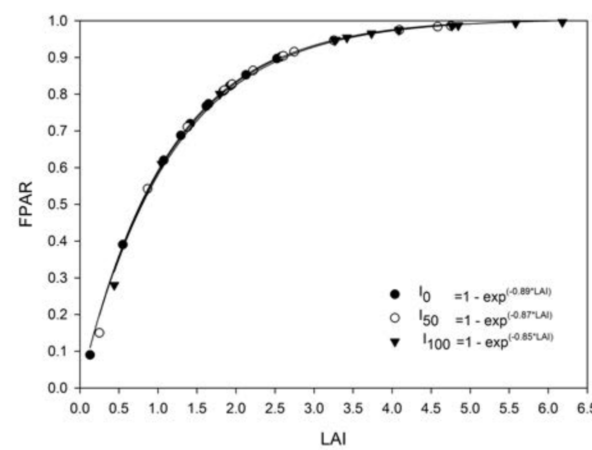

Figure 9. Relationship between periodic measurements of leaf area index (LAI) and the fraction of intercepted photosynthetically active radiation (FPAR) of *Saccharum spontaneum* L. ssp. *aegyptiacum* under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition).

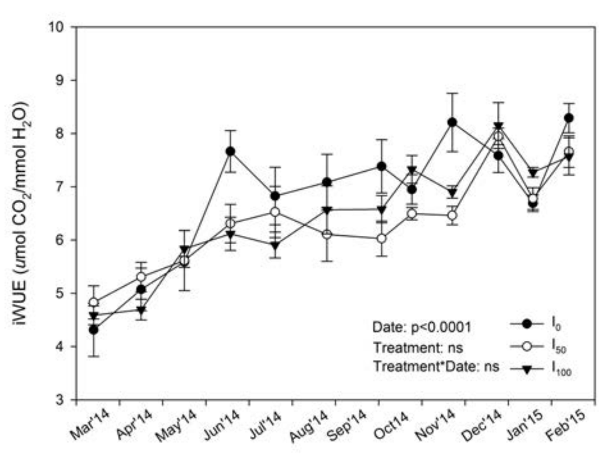

Figure 8. Instantaneous water use efficiency (μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) of *Saccharum spontaneum* L. ssp. aegyptiacum under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I_0 - rainfed condition) at different measurement time. Date represents the within-factor and irrigation the between-factor according to the GLM repeated measures ANOVA. iWUE, intrinsic water use efficiency.

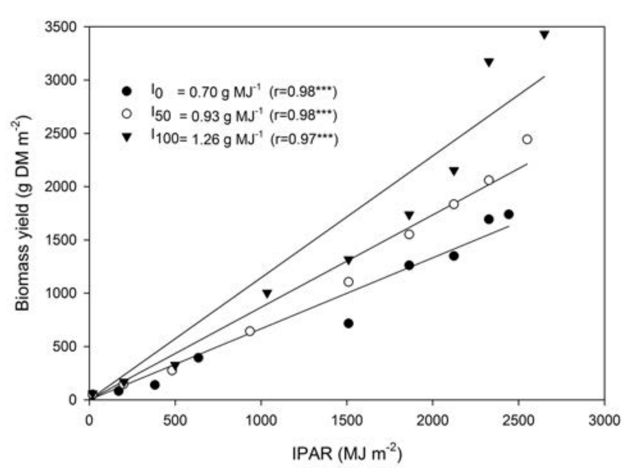

Figure 10. Relationships between intercepted photosynthetically active radiation (IPAR) (MJ m^{-2}) and aboveground biomass yield dry matter (g DM m^{-2}) of *Saccharum spontaneum* L. ssp. aegyptiacum under different soil water availability (I_{100} - 100% ETm restitution, I_{50} - 50% ETm restitution and I0 - rainfed condition). The slopes represent the radiation use efficiency (g MJ^{-1}). Pearson's correlation coefficients (r) at P≤0.05.

per unit water transpired (WUE) strength the idea of this species as candidate energy crop. As every undomesticated crop, however, further studies are needed from an agronomic point of view, such as timing and method of propagation, water and fertilization management, harvest time and post-harvest practices, as well as from technological, energetic and environmental point of view.

References

Angelini LG, Ceccarini L, Nassi o Di Nasso N, Bonari E, 2009. Comparison of Arundo donax L. and Miscanthus x giganteus in a long-term field experiment in Central Italy: analysis of productive characteristics and energy balance. Biomass Bioenerg. 33:635-43.

Arundale RA, Dohleman FG, Heaton EA, McGrath JM, Voigt TB, Long SP, 2014. Yields of Miscanthus x giganteus and Panicum virgatum decline with stand age in Midwestern USA. GCB Bioenergy 6:1-13.

Bunce JA, 1982. Low humidity effects on photosynthesis in single leaves of C4 plants. Oecologia (Berl.), 54:233-5.

Chaves MM, Flexas J, Pinheiro C, 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103:551-60.

Cosentino SL, 1996. Crop physiology of sweet sorghum (Sorghum bicolor L. Moench) in relation to water and nitrogen stress. pp 30-41 in Proc. First Eur. Seminar on Sorghum for Energy and Industry, Toulouse, France.

Cosentino SL, Copani V, Patanè C, Mantineo M, D'Agosta GM, 2008. Agronomic, energetic and environmental aspects of biomass energy crops suitable for Italian environments. Ital. J. Agron. 2:81-95.

Cosentino SL, Copani V, Testa G, Scordia D, 2015. Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. a potential perennial grass for biomass production in marginal land in semi-arid Mediterranean environment. Industr. Crops Prod. [In press].

Cosentino SL, Foti S, D'Agosta GM, Mantineo M, Copani V, 2005. Confronto tra gli impatti ambientali di biocombustibili e di combustibili fossili per mezzo della "Life Cycle Assessment" (LCA). Agroindustria 4:109-28.

Cosentino SL, Patanè C, Sanzone E, Copani V, Foti S, 2007. Effect of soil water content and nitrogen supply on the productivity of Miscanthus × giganteus Greef and Deu. in Mediterranean environment. Ind. Crop Prod. 25:75-88.

Cosentino SL, Scordia D, Sanzone E, Testa G., Copani V, 2014. Response of giant reed (Arundo donax L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment. Eur. J. Agron. 60:22-32.

Cosentino SL, Testa G, Scordia D, Alexopoulou E, 2012. Future yields assessment of bioenergy crops in relation to climate change and technological development in Europe. Ital J. Agron. 7:154-66.

Dohleman FG, Long SP, 2009. More productive than maize in the midwest: how does miscanthus do it? Plant Physiol. 150:2104-15.

El-Sharkawy AM, Cock JH, Del Pilar Hernandez A, 1985. Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. Photosyn. Res. 7:137-49.

Erice G, Louahlia S, Irigoyen JJ, Sanchez-Diaz M, Avice JC, 2010. Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. J. Plant Physiol. 167:114-20.

European Commission, 2009. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. In: Official Journal, L 140, 5/6/2009, pp 16-62.

Flexas J, Diaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbo M, 2007. Rapid variations of mesophyll conductance in response to changes in CO2 concentration around leaves. Plant Cell Environ. 30:1284-98.

Foti S, Cosentino SL, Patanè C, Copani V, Sanzone E, 2003. Plant indicators of available soil water in Miscanthus × giganteus Greef and Deu. Agronomie 23:29-36.

Grzesiak MT, Waligórski P, Janowiak F, Marci ska I, Hura K, Szczyrek P, Glab T, 2013. The relations between drought susceptibility index based on grain yield (DSIGY) and key physiological seedling traits in maize and triticale genotypes. Acta Physiol. Plant. 35:549-65.

Heaton EA, Dohleman FG, Long SP, 2008. Meeting us biofuel goals with less land: the potential of Miscanthus. GCB Bioenerg. 14:2000-14.

Heaton EA, Voigt T, Long SP, 2004. A quantitative review comparing the yields of two candidate C4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass Bioenerg. 27:21-30.

IPCC, 2013. Summary for policymakers. In: TF Stocker, D Qin, G-K Plattner, M Tignor, SK Allen, J Boschung, A Nauels, Y Xia, V Bex, PM Midgley (Eds.), Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, PAGES???

Italian Regulation, 2014. Ministero dello Sviluppo Economico, Decreto 10 ottobre 2014. Aggiornamento delle condizioni, dei criteri e delle modalita di attuazione dell'obbligo di immissione in consumo di biocarburanti compresi quelli avanzati. In: G.U. Ser. Gen., n. 250, 27/10/2014, pp 81-88.

Kiniry JR, Johnson MV, Bruckerhoff SB, Kaiser JU, Cordsiemon RL, Harmel RD, 2011. Clash of the titans: comparing productivity via radiation use efficiency for two grass giants of the biofuel field. Bioenergy Res. 5:41-8.

Kiniry JR, Landivar JA, Witt M, Gerik TJ, Cavero J, Wade LJ, 1998. Radiation-use efficiency response to vapor pressure deficit for maize and sorghum. Field Crop Res. 56:265-70.

Kiniry JR, Tischler CR, Van Esbroeck GA, 1999. Radiation use efficiency and leaf CO_2 exchange for diverse C4 grasses. Biomass Bioenerg. 17:95-112.

Meek DW, Hatfield JL, Howell TA, Idso SB, Reginato RJ., 1984. A generalized relationship between photosynthetically active radiation and solar radiation. Agron J. 76:939-45.

Monteith JL, 1965. Light distribution and photosynthesis in field crops. Ann Bot. 29:17-37.

Mott KA, Parkhust DF, 1991. Stomatal responses to humidity in air and helox. Plant Cell Environ. 14:509-15.

Nassi o Di Nasso N, Roncucci N, Triana F, Tozzini C, Ragaglini G, Bonari E, 2011. Productivity of giant reed (Arundo donax L.) and miscanthus (Miscanthus x giganteus Greef et Deuter) as energy crops: growth analysis. Ital. J. Agron. 6:141-7.

Scordia D, Cosentino SL, Jeffries TW, 2010. Second generation bioethanol production from Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. Bioresour. Technol. 101:5358-65.

Scordia D, Testa G, Cosentino SL, 2014. Perennial grasses as lignocellulosic feedstock for second-generation bioethanol production in Mediterranean environment. Ital. J. Agron. 9:84-92.

Stockle CO, Kiniry JR, 1990. Variability in crop radiation-use efficiency associated with vapor pressure deficit. Field Crops Res. 21:171-81.

Strullu L, Cadoux S, Preudhomme M, Jeuffroy MH, Beaudoin N, 2011. Biomass production and nitrogen accumulation and remobilization by Miscanthus × giganteus as influenced by nitrogen stocks in belowground organs. Field Crop Res. 121:381-91.

Zegada-Lizarazu W, Elbersen W, Cosentino SL, Zatta A, Alexopoulou E, Monti A, 2010. Agronomic aspects of future energy crops in Europe. Biofuels Bioprod. Bioref. 4:674-91.