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 area 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 (I100, I50 and I0, corresponding to 100%, 50% and 0% of ETm restitution) under a semi-arid Mediterranean environment. Leaf area index (LAI), stem height, biomass dry matter yield, CO₂ assimilation rate, and transpiration rate resulted significantly affected by measurement time and irrigation treatment, with the highest values in I100 and the lowest in I0. RUE was the highest in I100 followed by I50 and I0; on the other hand, WUE was higher in I0 than I50 and I100. At LAI values greater than 2.0, 85% photosynthetically active radiation was intercepted by the *Saccharum* stand, irrespective of the irrigation treatment.

*Saccharum spontaneum* ssp. *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.

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 area is strongly affected by prolonged summer drought which in turn limits yields of several crops. Furthermore, high vapour pressure deficit (VPD) reduces leaf conductance, affecting CO₂ 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 area, 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. aegypticum 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 a reduced 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 (Erico et al., 2010). Leaf area is the main determinant of the rate
of intercepted photosynthetically active radiation (IPAR). Understanding factors controlling leaf area and 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 effi-
ciency (NUE)] (Kiniry et al., 2011). In the present work we investigat-
ed the effect of soil water availability 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. aegypticum (Wild.). Hackel. Furthermore, the net increase of bio-
mass 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).

Rhizomes of Saccharum spontaneum L. ssp. aegypticum (Wild.)
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 m²
(5x3 m). Before transplanting 100 kg N ha⁻¹ and 100 kg P₂O₅ ha⁻¹ as
ammonium sulphate and supersphosphate, 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 estab-
lishment 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 evap-
orationtranspiration restitution (ETm): l₁₀₀ (100% ETm), l₁₀ (50% ETm) and
l₁ (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 coe-
efficients were as reported by Cosentino et al. (2015).

**Measurements**

Main meteorological parameters were measured by means of a weath-
er station connected to a data logger (CR10; Campbell Scientific, Logan,
UT USA), located 100 m from the experimental field. Gas exchange activ-
ities, as assimilation rate (A, μmol CO₂ m⁻² s⁻¹), transpiration rate (E,
mmol H₂O 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 CO₂ 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. Intrinsuc WUE (iWUE) was calculated as the ratio between net pho-
tosynthesis and transpiration rate at each measurement time (μmol
CO₂ mmol⁻¹ H₂O), 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 cal-
culated:

\[
\text{FPAR} = \frac{\text{PARin} - \text{PARs}}{\text{PARin}} \tag{1}
\]

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 PAR was described by an asymptotic equation [y = 1 –
e^{–kx/LAI}], where k represents the extinction coefficient. This formula
was used to calculate the PAR intercepted by the crop, assuming a lin-
ear behavior 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):

\[
\text{IPAR} = \sum_{i=1}^{n} \text{PARi} \times \text{FPARi} \tag{2}
\]

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 calcu-
lated by means of linear regressions. RUE values were the slopes of the
regressions of aboveground yield (g DM m⁻²) as a function of cumulat-
ed 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 inter-
vals). 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.

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 65°C and kept until constant weight, the
latter were used for LAI determination before to be dried as above.

Fresh green LAI was measured by means of a Delta-T Area
Measurement System (Delta-T Devices Ltd., Burwell, Cambridge,
England) as leaf area on ground area unit. The area meter was calibrat-
ed against paper standards of known area. Green LAI was accepted
when >50% green tissue was detected by visual score.
Statistical analysis

Data for 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 0.05 criterion). 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 the lowest in February (12.8 MJ m⁻² d⁻¹, as monthly averaged) and the 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. I₁₀₀ showed the highest values (218.2 cm), while I₀ the lowest (127.5 cm). I₅₀ was significantly different between both treatments, 206.4 cm (Figure 2). Leaf area index peaked at September 2014 (6.18, 4.75 and 2.58 in I₁₀₀, I₅₀ and I₀, respectively) and subsequently declined down to 2.49 (I₁₀₀), 1.70 (I₅₀) and 1.25 (I₀) at harvest time (February 2015), as shown in Figure 3. 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 I₁₀₀, 24.4 Mg DM ha⁻¹ in I₅₀ and 34.3 Mg DM ha⁻¹ in I₀. At harvest, DM yield slightly decreased down to 14.1, 20.8 and 28.0 Mg DM ha⁻¹ in I₁₀₀, I₅₀ and I₀, respectively (Figure 4).

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 the highest in I100, intermediate in I50 and was the lowest in I0. As result, the biomass yield as function of CWU led to significantly higher cWUE in I100 than I50 and I0 (6.55 vs 5.29 and 5.19 g L−1). On the other hand, the CRUE was significantly highest in I100 (1.29 g MJ−1), intermediate in I50 (0.96 g MJ−1) and the lowest in I0 (0.71 g MJ−1).

Crop physiology and intercepted photosynthetically active radiation

CO2 assimilation rate was similar before treatment differentiation (March-April 2014), afterwards it was highest in I100, followed by I50 with a peak on May 2014 (32.4 and 26.8 μmol CO2 m−2 s−1 in I100 and I50, respectively) (Figure 5). As the growing season approached summertime, and so temperatures and VPD increased, the assimilation rate of the crop decreased. Indeed, as illustrated in 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 among treatments: both watered treatments (I100 and I50) maintained lower leaf temperature than air temperature, while I0 showed higher leaf than air temperature on June, July and August. Hence, cumulated difference between leaf and maximum air temperature (Figure 6B) was positive in I0 from June to August (0.21°C to 3.15°C), while it was always negative in both I50 and I100.

The CO2 assimilation rate became similar between treatments from November 2014 onward.

Transpiration rate followed the same trend described for CO2 assimilation rate. Maximum transpiration rate in watered treatments was measured on May 2014 (5.5 and 4.8 mmol H2O m−2 s−1 in I100 and I50, respectively) and subsequently declined to match with the rainfed condition (I0) from December 2014 (Figure 7).

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 measurement times, 6.80 μmol CO2 mmol−1 H2O were found in I0, 6.34 μmol CO2 mmol−1 H2O in I50 and 6.45 μmol CO2 mmol−1 H2O in I100.

IPAR 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. I0 intercepted 90% PAR due to a maximum LAI of 2.58, while both I50 and I100 were able to intercept all the available PAR (100%) due to a greater LAI.

A different light extinction coefficient (k) was found among treatments: 0.89 in I0, 0.87 in I50 and 0.85 in I100 (Figure 9).

The net increase of biomass production (g m−2) per unit light intercepted (MJ m−2) led to the highest RUE in I100 (1.26 g MJ−1), followed by I50 (0.93 g MJ−1) and I0 (0.70 g MJ−1), as shown in Figure 10.

According to the Pearson’s test, high and positive correlation coefficients (P<0.0001) were found in all relationships between the IPAR and the aboveground DM yield (0.97 in I50 and 0.98 in both I0 and I100).

**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    | 6.55a        | 2442.6        | 0.71c         |
| I50       | 461.6    | 5.29b        | 2550.3        | 0.96b         |
| I100      | 661.6    | 5.19b        | 2650.1        | 1.29a         |

*a,b Different letters in the same column indicate statistical significance according to Bonferroni test at P<0.05.*
Discussion

It has been previously shown that Saccharum spontaneum spp. aegyptiacum possesses a range of agronomic, physiologic and qualitative desirable traits of biomass crop, namely C4 plant, high biomass yield, high water efficiently, able to assimilate CO2 during drought-stress periods, high cellulose and hemicellulose content (Scordia et al., 2010, 2014; Cosentino et al., 2015).

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.

CO2 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 Parkhurst, 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 keep green LAI up to harvest time (1.25, 1.70 and 2.49, I0, I50 and I100, 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) showed that Saccharum yields were 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; Dolehman 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 L of water in Saccharum I100 and 6.5 g in Saccharum L. Miscanthus and 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 I3 and I100 treatments, while in rainfed condition (I0) the values matched well between the two calculation methods (6.80 μmol CO2 mmol−1 H2O. and 6.55 g L−1, respectively). Both RUE and cRUE were the highest in the full-watered, followed by the half-watered 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 × 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, as it does for CO₂ assimilation rate (Kiniry et al., 1998). For instance, Bunce (1982) found that maize CO₂ 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 CO₂ 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) showed a liner decrease of RUE as a 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, having also a greater impact on both CO₂ 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 underestimate RUE. 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 CO₂ assimilation, the high net increase of biomass production per unit light intercepted (RUE) and per unit water transpired (WUE) strength the idea of this species as candidate energy crop. As 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.

Figure 8. Instantaneous water use efficiency (μmol CO₂ mmol⁻¹ H₂O) of Saccharum spontaneum L. ssp. aegyptiacum under different soil water availability (I100 - 100% ETm restitution, I50 - 50% ETm restitution and I0 - 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 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 (I100 - 100% ETm restitution, I50 - 50% ETm restitution and I0 - rainfed condition).

Figure 10. Relationships between intercepted photosynthetically active radiation (IPAR) (MJ m⁻²) and aboveground dry matter yield (g DM m⁻²) of Saccharum spontaneum L. ssp. aegyptiacum under different soil water availability (I100 - 100% ETm restitution, I50 - 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.
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