THE RESPONSE OF LIGNOCELLULOSIC PERENNIAL GRASSES TO DIFFERENT SOIL WATER AVAILABILITY

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ABSTRACT: The Mediterranean climate is a climate change hot spot suffering by increasing drought periods during summer, with low rainfall and high evapotranspiration, which are limiting conditions for plant CO_2 assimilation and biomass production, particularly for spring-summer crops. This would be even worst under marginal lands, where climatic, terrain and other limiting factors will further aggravate growing conditions and productivity. The present field experiment investigated the response to irrigation of several lignocellulosic perennial grasses. Plant stem density, stem height, stem weight and leaf area index were evaluated during the growing season with a monthly frequency. Stem density showed the lowest values for *A. donax* genotypes and the highest for the *Miscanthus* seed-based hybrids. High stem densities are observed at high irrigation levels. Stem height and weight are inversely correlated to stem density. *A. donax* genotypes had higher stem height and weight than the other genotypes. Irrigation has a significant positive effect on both measures. Leaf area index varied among genotypes, and irrigation increased this trait.
Keywords: biomass, bioenergy, perennial energy crops, lignocellulosic, marginal land.

1 INTRODUCTION

The field experiment investigated the response to irrigation of several genotypes for which data is not available in the scientific literature: Miscanthus seed-based hybrids GNT9 and GNT10 have been selected for the adaptability to drought conditions among the F1 generation obtained from interspecific cross breeding of Miscanthus. These are compared with the endemic perennial grasses most promising under drought conditions in the semiarid Mediterranean environments [1].

The Mediterranean climate is characterized by long drought periods during summer, with low rainfall and high evapotranspiration, which are limiting conditions for plant CO_2 assimilation and biomass production, particularly for spring-summer crops. In this environment, water scarcity limits crop yields, particularly of spring-summer crops. Climate change scenarios predict prolonged summer drought episodes, with higher air temperature, higher evapotranspiration and lower frequency of rainfall [2]. The increasing aridity and thus marginality leads to agricultural land abandon [3]. Marginal lands are the primary lands intended for energy crops io order to avoid the competition with food crops. The thresholds to define marginal lands in terms of biophysical constraints have been set by Joint Research Centre (JRC) of the European Commission [4]. The focus of the present study is toward the marginality due to the climate limitation given by the ratio between precipitations and potential evapotranspiration (P/PET). Areas with P/PET < 0.6 are classified as affected by dryness.

Water and energy efficiency in agriculture is a main goal of agricultural research with the aim of reducing crop water footprint and external energy requirement per unit of production, contributing to reduce the pressure on the limited global freshwater resources and non-renewable energy sources [5]. Perennial crops are recognised to be more efficient than annual crops concerning the biomass production. Among them, the perennial bioenergy grasses giant reed (*Arundo donax* L.), miscanthus (*Miscanthus* x *giganteus* (Greef et Deuter) and African fodder cane (*Saccharum spontaneum* L. ssp. *aegypticum* Wild (Hack.) are selected as the most suited to Mediterranean conditions [1].

Giant reed, native from Great Middle East and African fodder cane, native from North Africa are naturalized in the warmest area of the Mediterranean basin. *Miscanthus × giganteus*, is a hybrid of *M. sinensis* and *M. sacchariflorus*, native of Eastern Asia but well adapted to European cool-temperate climate [6]. New hybrids have been developed to cope with the low water availability of southern Europe [7].

African fodder cane and miscanthus have a C4 pathway, while giant reed has a C3 pathway [8, 9]. They share a high biomass yield, the ability to use water efficiently and satisfactory biomass quality; and therefore hold a great promise for biomass production in drought affected areas [10].

The field trial aims to assess the response of diverse lignocellulosic perennial grasses under three different irrigation regimes in the second year after plant establishment. Plant stem density, stem height, stem weight and leaf area index were evaluated during the growing season with a monthly frequency.

2 MATERIAL AND METHODS

2.1 Field trial description

The field trial was carried out at the Experimental Farm of the University of Catania (10 m a.s.l., 37°24′ N, 15°03′ E) in a typical Xerofluvents soil (USDA, 1999).

Six genotypes were evaluated in a split-plot experimental design with nine replications: two *A. donax* L. ecotypes, named ARCT and ARMO (clone Fondachello and clone Morocco), the commercial *Miscanthus* x *giganteus* (Greef et Deuter) named MxG, two seed-based *Miscanthus* hybrids obtained from the breeding program led by the Institute of Biological, Environmental and Rural Sciences of Aberystwyth University (UK) and Terravesta Ltd (UK), named GNT9 and GNT10, and one ecotype of *S. spontaneum*, named SAC. The main factor assigned to the plots is the irrigation factor, with 3 levels: 100%, 50% and 0% of maximum crop evapotranspiration (ETm) restoration during the summer months (June-August).

Genotype is the second factor, assigned to the sub-plots within the main irrigation plots. Each combination of irrigation and genotype is replicated 3 times within the main plots.

Irrigation is provided by a drip irrigation system. During the first year of plant establishment, irrigation was not differentiated, and all the plots received 100% of maximum crop evapotranspiration restoration during the summer months.

Rhizomes of SAC, ARMO and ARCT were collected from the in-situ germplasm collection located at the Experimental farm. Plantlets of MxG were provided by Energene sp. z o.o (Poland), while the GNT9 and the GNT10 by Terravesta Ltd (UK). Fresh rhizomes of approximately 100 g with 2 - 3 main buds (ARMO, ARCT and SAC) and plantlets (MxG, GNT9 and GNT10) were directly transplanted in a previously prepared soil bed, ploughed in autumn, and disk harrowed in spring. Transplant was done by hand in May 2018 at a density of 1 rhizome or plant m^{-2}. With the aim to reduce the external input supply, no fertilization was supplied before transplanting,

Weeds were controlled manually during the year of establishment by means of a grass trimmer when necessary. Plantlets were kept in well-watered condition through a drip irrigation system, from the establishment to the end of summertime, by restoring 100% ETm.

Irrigation was scheduled when the sum of daily ETm corresponded to the volume, subtracting rainfall events from the calculation. The daily ETm was calculated according to:

$$ETm = ET0 \times Kc$$

where ETm is the maximum daily evapotranspiration (mm); E0 is the evaporation of class-A pan (mm); Kp is the pan coefficient, equal to 0.80 in semi-arid environment. Crop coefficients (Kc) were those applied for *Miscanthus* × *giganteus*, *Arundo donax* and *Saccharum spontaneum* grown in the same environment [8, 10, 11].

The irrigation volume was calculated according to the following equation:

$$V = 0.66 \times (FC - WP) \times \phi \times D \times 10^3$$

where V = water amount (mm); 0.66 = readily available water not limiting for evapotranspiration; FC = soil water content at field capacity (27% of dry soil weight); WP = soil water content at wilting point (11% of dry soil weight); ϕ = bulk density (1.1 g cm^{-3}); and D = rooting depth (0.6 m).

At the end of summer season, when rainfall increases in frequency, the irrigation was suspended.

2.2 Measurements

Field measurements of stem density have been measured monthly from shoot emission at the beginning of spring to leaves senescence in autumn, counting the number of stems within the inner 4 m^2 subplot.

Stem sampling has been performed monthly starting from shoot emission until harvest in winter. Stem sampling consist of the collection of one (ARMO and ARCT) or 3 (all the other genotypes) representative stems from each plot of the trial, avoiding the inner 4 m^2 subplot that has been used for the estimation of total aboveground biomass yield at winter harvest.

The sampled stems have been measured for stem height (m), green leaf area per stem (cm^2), fresh and dry stem weight (g), the latter obtained by drying the biomass at 65°C until constant weight. Leaf area index has been calculated by multiplying stem density and leaf area per stem.

2.4 Statistical analysis

Stem height, dry weight, stem density and LAI were subjected to a three-factor ANOVA using repeated measure on time, with date, genotype and the irrigation factor as the sources of variance using R software [12].

3 RESULTS

3.1 Meteorological conditions

During the growing season air temperature gradually increased from the time of transplant to peak in summer and then slowly decreased in autumn down to the lowest values in the winter. In spring (March, April, May), the average minimum air temperature was 9.7°C and the maximum 20.5°C. In summer (June, July, August), the minimum air temperature was 19.5°C and the maximum 31.6°C. In autumn (September, October, November), the minimum and the maximum air temperature were 15.2 and 25.4°C, respectively. The mean temperature in winter was higher than 8°C (11.0°C), anyway always higher than 3.9°C for the minimum. Rainfall over the whole season was close to the average for this location. The cumulative rainfall for the year was 572.8 mm, however, the distribution was uneven, with most of the events concentrated in autumn. Spring and summer cumulative rainfall was 78.2 mm, while that of autumn was 375 mm, with a few events of very high intensity in October and November. On the contrary, the reference evapotranspiration (ET0) was lower in autumn (229 mm), than spring-summer (653 mm) which corresponds to the maximum crop vegetative development and highest crop water requirement.

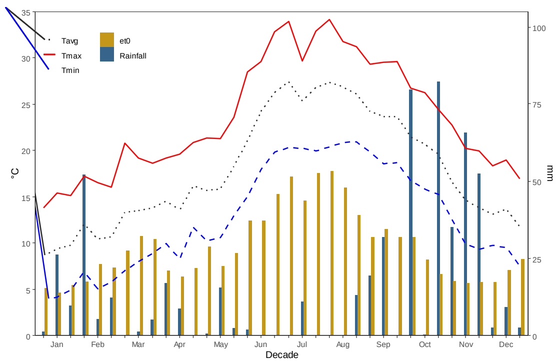


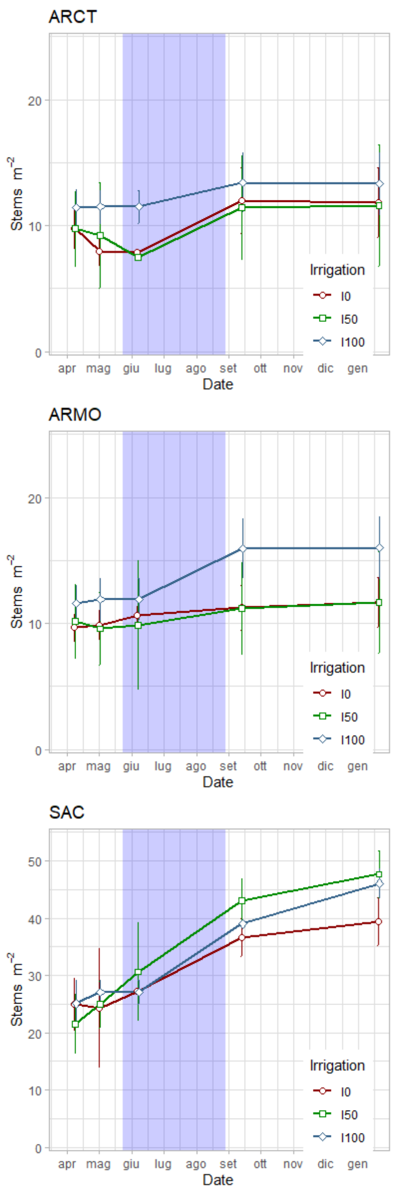
Figure 1: Main meteorological parameters registered in Autumn and Winter growing season at the Experimental farm of the University of Catania, Italy (37°25' N., 15°03' E., 10 m a.s.l.).

3.2 Crop measurements

The ANOVA showed significance of main effects and interaction on morphological traits analyzed (Table 1). Genotype GNT9 had the highest stem density, peaking at 85 stems m^{-2} in the I100 (Figure 2). Both *A. donax* genotypes had the lowest stem density among the six genotypes for all the irrigation levels. Generally, stem density increases with the higher irrigation levels, however some exceptions have been reported. Date, genotype and irrigation have a statistically significant effect on stem density, while among the interactions, only date x genotype and irrigation x genotype have a statistically significant effect (data not shown). All the genotypes reached the maximum stem density at the end of summer or during autumn, excluding GNT9 that reached the maximum stem density in May and showed a decrease during summer.

Table I: ANOVA of the main factors and interactions on morphological traits perennial grasses under different irrigation and measurement time (significance level at P≤0.05). SD: stem density; DW: stem dry weight; SH: stem height, LAI: leaf area index

	SD	DW	SH	LAI
Date (D)	≤0.001	≤0.001	≤0.001	≤0.001
Genotype (G)	≤0.001	≤0.001	≤0.001	≤0.001
Irrigation (I)	≤0.001	≤0.001	≤0.001	≤0.001
D x I	ns	≤0.001	≤0.001	≤0.001
D x G	≤0.001	≤0.001	≤0.001	≤0.001
I x G	≤0.001	≤0.001	≤0.001	≤0.001
D x I x G	ns	≤0.001	ns	≤0.001



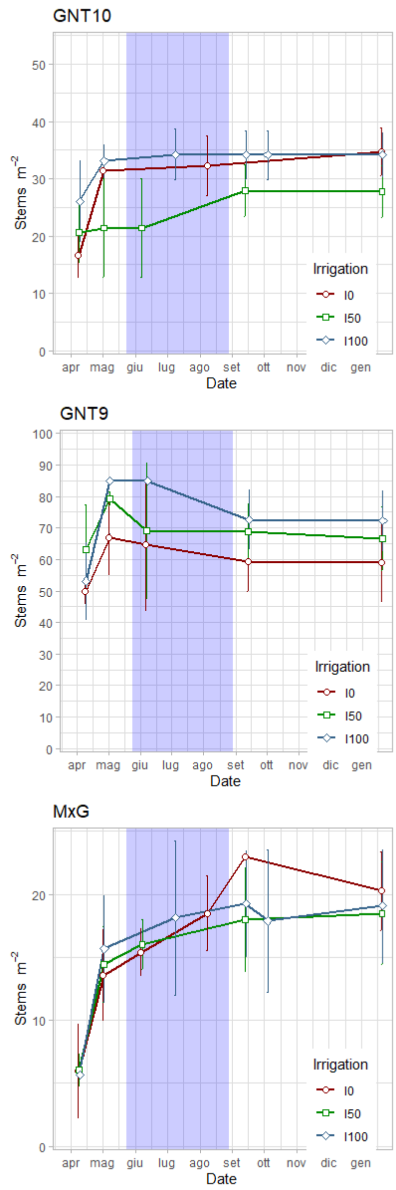
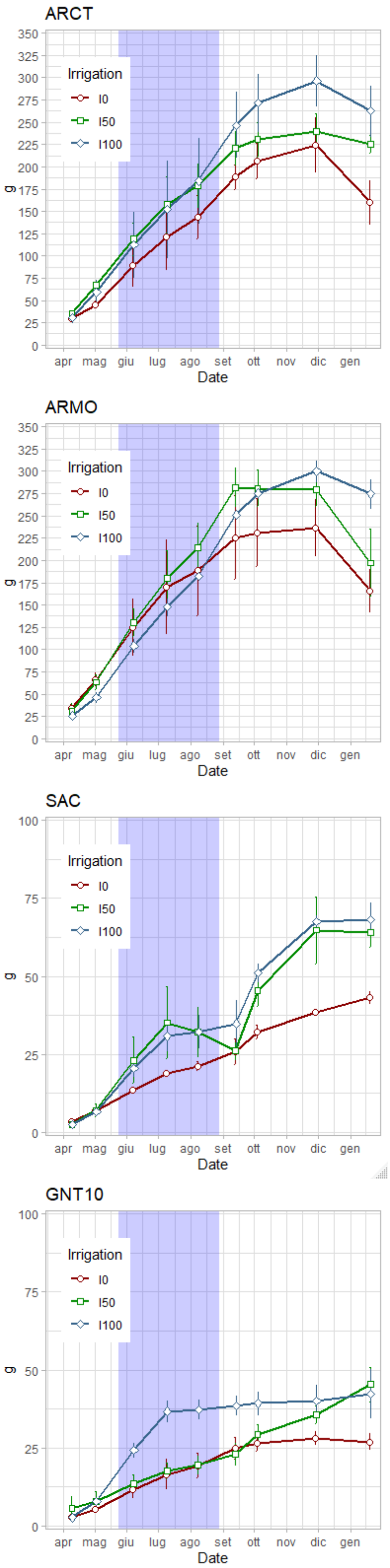


Figure 2: Stem density (stems m^{-2}) trends over the vegetative stages (March to November 2019) for 3 water restoration levels (I0, I50, I100, irrigation interval in purple) and for 6 genotypes exanimated in this study: ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* x *giganteus* hybrid 10, GNT9: *Miscanthus* x *giganteus* hybrid 9, MxG: *Miscanthus* x *giganteus.*

Mean stem dry weight showed a high variability among genotypes (F-value of 1830), with values ranging from 302 g for ARMO to 13.5 g for MxG in the I100 treatment (Figure 3). *A. donax* genotypes showed the highest stem dry weight during the whole growing season for all the irrigations. *S. spontaneum* and GNT10 showed intermediate values, while GNT9 and MxG had the lowest stem dry weights. Irrigation has a statistically significant positive effect on stem dry weight. Date, genotype, irrigation and all factors' interactions have a statistically significant effect on stem height (data not shown).



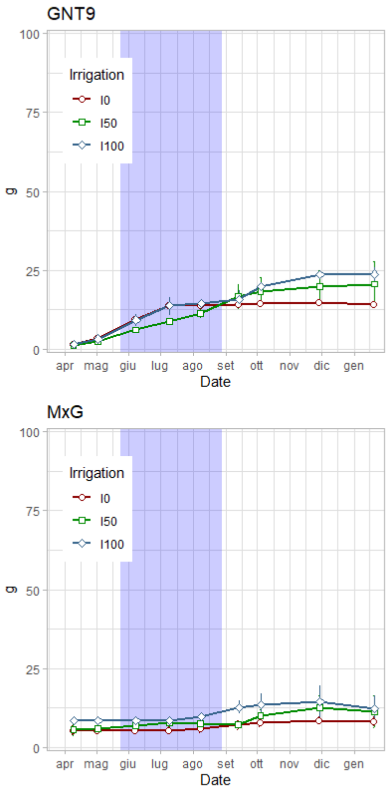
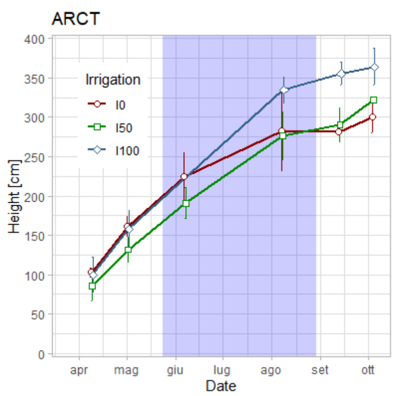
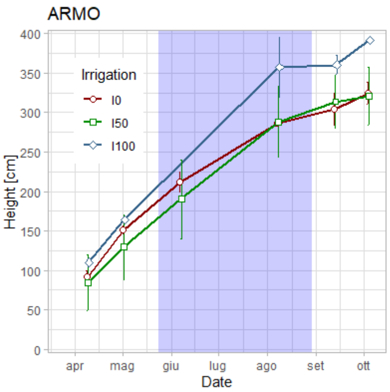
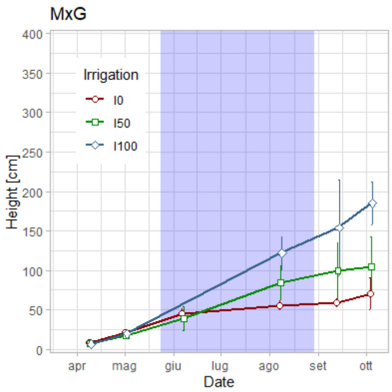


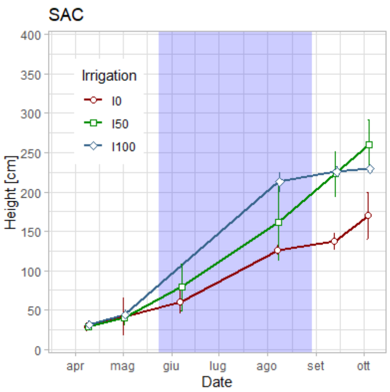
Figure 3: Mean stem dry weight (g stems^{-1}) trends over the vegetative stages (March to November 2019) for 3 water restoration levels (I0, I50, I100, irrigation interval in purple) and for 6 genotypes examinated in this study: ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* x *giganteus* hybrid 10, GNT9: *Miscanthus* x *giganteus* hybrid 9, MxG: *Miscanthus* x *giganteus*.

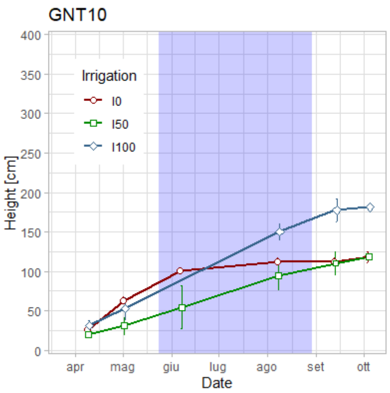
A. donax genotypes had the highest stem height, with ARMO peaking at almost 400 cm, followed by *S. spontaneum*, while *Miscanthus* genotypes had the lowest stem height (Figure 4). Irrigation has a statistically significant positive effect on stem height, with I00 treatment showing higher stems for all the genotypes and I0 treatment showing the lowest. Date, genotype, irrigation and all factors' interactions have a statistically significant effect on stem (data not shown). Stem height increased continuously until late autumn in all the genotypes.











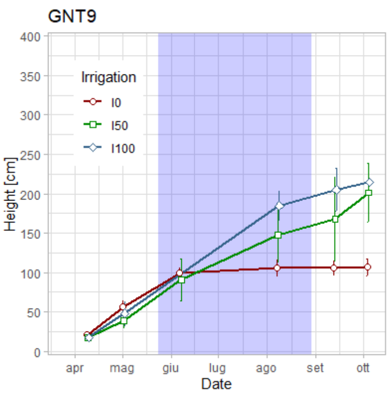
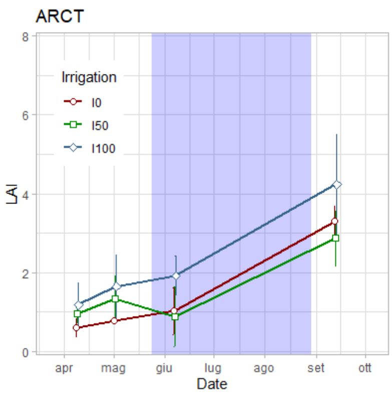
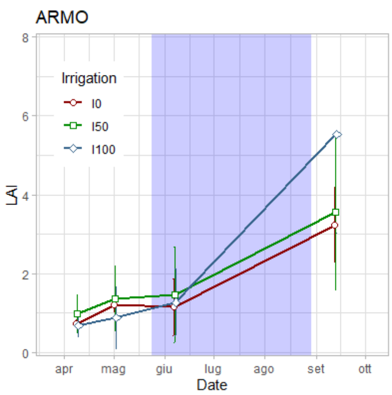


Figure 4: Mean stem height (stems m^{-2}) trends over the vegetative stages (March to November 2019) for 3 water restoration levels (I0, I50, I100, irrigation interval in purple) and for 6 genotypes examinated in this study: ARCT: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* x *giganteus* hybrid 10, GNT9: *Miscanthus* x *giganteus* hybrid 9, MxG: *Miscanthus* x *giganteus*.

Values of LAI reached a maximum of 5.7 in ARMO I100 (Figure 5). Maximum LAI has been reached between September and October for all the genotypes. ANOVA was statistically significant for date, genotype, irrigation and all factors' interactions (data not shown).





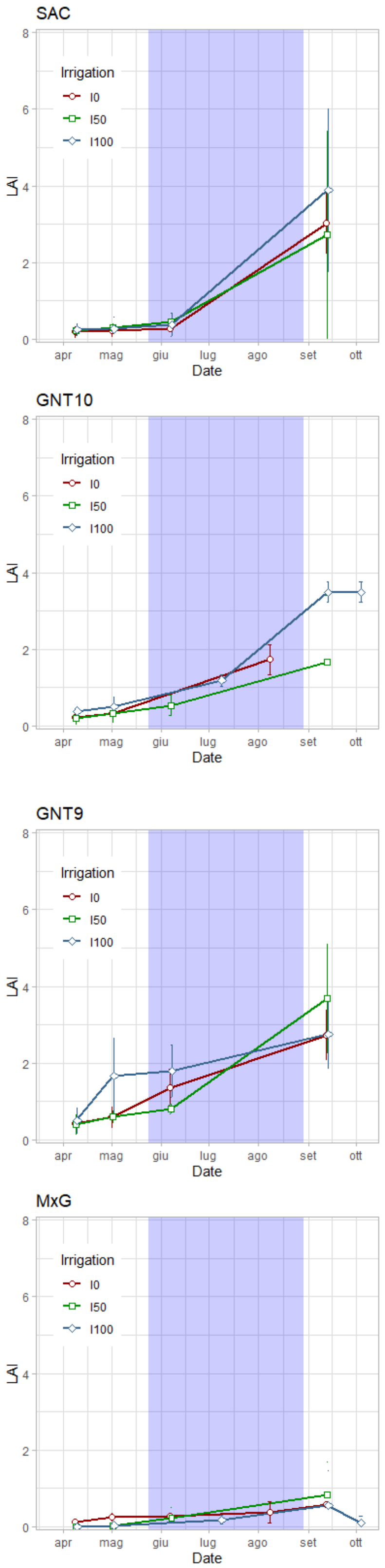


Figure 5: Leaf area index trends over the vegetative stages (March to November 2019) as calculated by measuring stem leaf area per stems and stem density for 3 water restoration levels (I0, I50, I100, irrigation interval in purple) and for 6 genotypes exanimated in this study: *A. donax* ecotype Catania, ARMO: *A. donax* ecotype Morocco, SAC: *S. spontaneum*, GNT10: *Miscanthus* x *giganteus* hybrid 10, GNT9: *Miscanthus* x *giganteus* hybrid 9, MxG: *Miscanthus* x *giganteus*.

4 DISCUSSION

Stem density, stem height and stem weight accounts for the greatest part of the biomass yield. In the present experiment, stem density significantly varied among genotypes, with the lowest values for *A. donax* genotypes and the highest for the *Miscanthus* seed-based hybrids. Generally, stem density increases with the higher irrigation levels, however some exceptions have been reported. Stem height and weight are inversely correlated to stem density. *A. donax* genotypes had higher stem height and weight than the other genotypes. Irrigation has a significant positive effect on both measures. Leaf area index varied among genotypes, and irrigation increased this trait. However, a different response was observed among genotypes, indicating a different adaption to drought.

5 CONCLUSIONS

The field experiment investigated the response to irrigation of several genotypes for which data is not available in the scientific literature: *Miscanthus* seed-based hybrids GNT9 and GNT10 have been selected for the adaptability to drought conditions among the F1 generation obtained from interspecific cross breeding of *Miscanthus*. These are compared with the endemic perennial grasses most promising under drought conditions in the semiarid Mediterranean environment.

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7 REFERENCES

[1] Scordia, D., Cosentino, S.L., 2019. Perennial Energy Grasses: Resilient Crops in a Changing European Agriculture. Agriculture, 9(8), 169.

[2] Cosentino SL, Testa G, Scordia D, Alexopoulou E. Future yields assessment of bioenergy crops in relation to climate change and technological development in Europe. Ital J Agronomy. 3 maggio 2012;7[2]:22.

[3] Panoutsou C, Chiaramonti D. Socio-Economic Opportunities from Miscanthus Cultivation in Marginal Land for Bioenergy. Energies. 29 maggio 2020;13[11]:2741.

[4] European Commission. Joint Research Centre. Scientific contribution on combining biophysical criteria underpinning the delineation of agricultural areas affected by specific constraints: methodology and factsheets for plausible criteria combinations. [Internet]. LU: Publications Office; 2014 [citato 28

aprile 2021]. Available at: https://data.europa.eu/doi/10.2788/844501.
[5] Scordia, D., Calcagno, S., Piccitto, A., Patanè, C., Cosentino S.L., 2020 The Impact of Soil Water Content on Yield, Composition, Energy, and Water Indicators of the Bioenergy Grass Saccharum spontaneum ssp. aegyptiacum under Three-Growing Seasons. Agronomy, 10, 1105.
[6] Clifton-Brown J, Hastings A, Mos M, McCalmont JP, Ashman C, Awty-Carroll D, et al., 2017. Progress in upscaling Miscanthus biomass production for the European bio-economy with seed-based hybrids. GCB Bioenergy 9(1), 6–17.
[7] Clifton-Brown, J., Schwarz, K.U., Awty-Carroll, D., Iurato, A., Meyer, H., Greef, J., Gwyn, J., Mos, M., Ashman, C., Hayes, C., et al., 2019 Breeding strategies to improve Miscanthus as a sustainable source of biomass for bioenergy and biorenewable products. Agronomy, 9, 673.
[8] Cosentino SL, Copani V, Testa G, Scordia D. Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. a potential perennial grass for biomass production in marginal land in semi-arid Mediterranean environment. Industrial Crops and Products. 2015;75:93–102.
[9] Cosentino, S.L., Patanè, C., Sanzone, E., Testa, G., Scordia, D., 2016. Leaf gas exchange, water status and radiation use efficiency of giant reed (Arundo donax L.) in a changing soil nitrogen fertilization and soil water availability in a semi-arid Mediterranean area. European Journal of Agronomy, 72, 56–69
[10] Cosentino SL, Scordia D, Sanzone E, Testa G, Copani V. Response of giant reed (Arundo donax L.) to nitrogen fertilization and soil water availability in semi-arid Mediterranean environment. European Journal of agronomy. 2014;60:22–32.
[11] Cosentino, S.L., Patane, 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
[12] R Core Team. R: A language and environment for statistical computing. R Core Team; 2013.